

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 ACTIVITY¹

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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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Lineweaver (3). With heated samples the pH changes occurred very slowly.

The test developed and patented by J. W. Stevens (4) was tried with the kind permission of the California Fruit Growers Exchange. This test involves the addition of barium chloride, pectin, a preservative and pH adjustment to 2.9-3.0. The samples were placed at 37°C. for 72 hours and then centrifuged. A clear supernatant liquid indicates enzyme activity. This test was satisfactory in many respects, but the elapsed time involved (72 hours) was objectionable and difficulty was experienced in judging borderline samples.

The next test was that described by Kertesz (5). This test had been devised for determining pectinesterase activity in tomato juice and involves the addition of methyl red indicator, and just sufficient alkali to cause the red color of the indicator to disappear. The reappearance of the red color was the indication of pectinesterase activity. This test worked very slowly in fresh orange juice and was not suitable as an indication of inactivation of the enzyme.

The use of a colored pH indicator has some advantages in that no elaborate equipment is required, so an effort was made to modify the test. The amount of enzyme was increased by using more juice relative to the amount of pectin. Equal volumes of juice and 1 percent pectin solution were used. This improved the test but it was still too slow to be useful. MacDonnell, Jansen and Lineweaver (3) reported that this enzyme was largely associated with the suspended matter in citrus juices and used a buffer containing sodium chloride or sodium acetate to bring the enzyme into solution. This provided the suggestion that sodium chloride be used to bring the enzyme into solution so that it would act more rapidly. With the addition of sodium chloride, encouraging results were obtained and definite changes in the color

were soon noticeable after adjusting the pH. However, Lineweaver and Ballou (2) had noted that the addition of suitable amounts of divalent cations (calcium or magnesium salts) resulted in an increase in pectinesterase activity. MacDonnell, Jansen and Lineweaver (3) showed that "by the addition of suitable amounts of various salts, the activity of orange pectinesterase was increased five-fold at pH 7.5 and more than 100-fold at pH 5." The use of calcium or magnesium chloride with the other reagents mentioned resulted in a decided stimulation in the enzyme activity. When salts were applied in amounts suggested by MacDonnell, Jansen and Lineweaver (3) to citrus juices even with very low pectinesterase activity, the red color returned in four hours or less. With fresh juice the change was evident within ten minutes.

The effect of temperature was observed by dividing the reaction mixtures into two portions in test tubes, placing one at 40° F and the other in crushed ice. The samples held at the higher temperature turned red sooner than the refrigerated samples. However, as might have been expected from the literature (2) the effect was not great and since one of the objects was to keep the test as simple as possible, it was decided that room temperature would be used and this was found to work very well.

An effort was made to use a mixed indicator. It was thought a combination of methyl red and methylene blue (Johnson and Green (6)) might improve the sensitivity of the test by providing a striking color change. The color change was from very light green to purple and very spectacular, but the test would not work. Apparently methylene blue inhibits the action of this enzyme.

No buffers were added or needed. Citrus juices are well buffered naturally.

The method we have found most satisfactory permits all the reagents to be

added in two solutions. As many as possible of the reagents were combined into one solution. This solution contained the pectin, sodium chloride, calcium or magnesium chloride, and methyl red. Sodium benzoate added to this solution will retard microbial growth but will not prevent degradation of the indicator. The main solution is prepared as follows:

Solution A

To one liter of distilled water, 12 grams of 150 grade pectin are added and thoroughly mixed in a Waring blender. A little of the pectin sticks to the jar so the resulting solution is about 1% pectin. Then 17.54 grams of sodium chloride are added (0.3 M) and 14.7 grams of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.1 M). To this 3 ml. of a freshly prepared 1% alcoholic solution of methyl red indicator are added. The solution is kept in the refrigerator.

Solution B

The other reagent is approximately 1.0 normal sodium hydroxide solution (40 g./liter).

In performing the test an equal quantity (20 ml.) of canned citrus juice and the reagent are mixed. The 1.0 normal sodium hydroxide solution is added with a medicine dropper with adequate mixing until the red color just disappears. By using approximately 1 N NaOH less dilution is obtained than with .1 or .01 N alkali. Citrus juice is naturally buffered so that no difficulty is experienced in obtaining suitable pH adjustment with the stronger reagent. The sample is then observed for the return of the red color. If pectinesterase is active, methyl ester groups are hydrolysed in the pectin, acid groups exposed, and the solution turns more acid, changing the indicator back to red. In fresh juices the color change is sufficient to be noticed in about fifteen minutes. With heated juices up to four hours have been observed to elapse be-

fore the color develops. When the enzyme has been inactivated there is no color change.

As a confirming test for the presence of the pectinesterase enzyme, the samples can be stoppered and placed in the refrigerator overnight. If there is much enzyme activity, a solid gel will form. In borderline cases lumps will form which can be observed by slowly inverting the flask. This confirmatory test is at least as sensitive as the color change, the only objection being that several hours are required for the setting of the gel or forming the lumps.

If a pH meter is available, changes in pH can, of course, be detected more satisfactorily with it than with the color indicator, but such an instrument is not essential.

The test has been applied to several hundred samples. Comparisons have been made with the Stevens (4) test. In the experimental work mentioned at the start of this paper, time and temperature of pasteurization tests were made. The temperature intervals were 5° F. Both the new test and the Stevens test were applied to these series and temperature of enzyme inactivation was determined. In one series, enzyme inactivation at 5° higher temperature was indicated by the new test. In fifteen series the results were identical. In twenty-seven series of samples the new test indicated inactivation temperatures 5° lower than the Stevens test. In one series the new test indicated a 10° lower inactivation temperature. In sixteen series the new test indicated the temperature of inactivation and the Stevens test gave borderline or indecisive results. The new test indicates temperatures of enzyme inactivation averaging less than 3° lower than the Stevens test. A complete listing of the inactivation temperatures along with a discussion of the factors affecting this temperature is to be presented in another paper.

Since the temperature needed for enzyme inactivation is above that used to pasteurize citrus juices, it is suggested that this rapid test for pectinesterase inactivation be used as an indication that adequate pasteurization temperatures have been used.

Summary

The new procedure has been devised and described for testing the effectiveness of the pasteurization of citrus juices. Evidence of the presence of pectinesterase is used as the index. The test is rapid in that it can be completed in four hours. A color indicator is used to detect changes in acidity in the prepared sample. The test is simple, requiring only two solutions, and can be easily performed in the control laboratory. A confirmation of the test can be

obtained by setting the prepared sample in the refrigerator overnight. The test agrees well with the results of other tests.

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STORAGE CHANGES IN FROZEN CONCENTRATED CITRUS JUICES—PRELIMINARY REPORT^{1 2}

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The purpose of this investigation, started in the 1949-50 season and now in progress, is to determine what effect temperature of storage will have on the quality of frozen concentrated citrus juices. In order that high quality may be retained, frozen citrus concentrates should be stored at temperatures suffi-

ciently low so that any chemical, microbiological, or enzymatic changes that may cause deterioration will be prevented or kept at a minimum.

Curl (3) studied the effects of degree of concentration and of temperature of storage on various characteristics of orange juices, that had been pasteurized prior to concentration and subsequently benzoated and stored at temperatures of 40° F. and above. Cotton and associates (2) made studies on frozen orange and tangerine concentrates stored at various temperatures. They reported excellent retention of aroma, taste, ascorbic acid and "cloud during storage at 0° F., but storage at 40° F. resulted in clarification, separation and flavor degradation. Rouse (4) presented data on gel formation in frozen citrus concentrates that had been thawed and stored at 40° F. His data indicated that the presence of low

¹ Cooperative research by the Florida Citrus Commission and the Florida Citrus Experiment Station.

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