1978

PASADENA CALIFORNIA

CITRUS RESEARCH CONFERENCE

On January 24, 1978, four USDA agencies--Agricultural Research Service (ARS), Cooperative State Research Service (CSRS), Extension Service (ES), and the National Agricultural Library (NAL)--merged to become a new organization, the Science and Education Administration (SEA), U.S. Department of Agriculture.

This publication was prepared by the Science and Education Administration's Agricultural Research staff, which was formerly the Agricultural Research Service.

CITRUS RESEARCH CONFERENCE

November 29, 1978

Pasadena, California

ABSTRACTS OF PAPERS

Sponsored By:

Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue
Pasadena, California 91106

Agricultural Research
Science and Education Administration
UNITED STATES DEPARTMENT OF AGRICULTURE

FOREWORD

This Citrus Research Conference is being held to bring to members of the citrus and allied industries in southern California and Arizona the latest results of research on the chemistry, pharmacology, and technology of citrus fruits and their products carried on by Agricultural Research, Science and Education Administration, U.S. Department of Agriculture. The following are participating in or contributing to this year's conference:

Western Region

Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue, Pasadena, California 91106

U.S. Horticultural Field Station
2021 South Peach Avenue, Fresno, California 93727

Southern Region

U.S. Citrus and Subtropical Products Laboratory
600 Avenue S, N.W., Winter Haven, Florida 33880

Food Crops Utilization Research Laboratory
Weslaco, Texas 78596

Conference Headquarters:

Huntington-Sheraton Hotel
1401 South Oak Knoll Avenue
Pasadena, California 91109

P R O G R A M

CITRUS RESEARCH CONFERENCE

Wednesday, November 29, 1978

MORNING SESSION - 9:00 A.M. Abstract on page

INTRODUCTORY REMARKS: Edward B. Knipling, Area Director
California-Hawaii-Nevada Area
AR-SEA-USDA, Fresno, California

RESEARCH TRENDS: Vincent P. Maier, Research Leader
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

AFTERNOON SESSION - 1:30 P.M.

RESEARCH ON BIOREGULATORS IN CITRUS*

Henry Yokoyama, Wan-Jean Hsu, Stephen M. Poling
and Ernest P. Hayman
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

In past years, research on the enhancement of color and provitamin A in citrus by various classes of bioregulators was reported. This work is based on the recognition that all plants including citrus have a great deal of unused biological potential. Agricultural research, until now, has been primarily concerned with increasing crop quality and yields by use of pesticides, fertilizers, irrigation and better management, coupled with variety development and genetic improvements; however, little attention has been given to the control of biological processes that limit crop quality and productivity. The unused biological potential is there to be tapped for increasing the quality, yields and stability of agricultural crop output.

We are concerned with the regulation of the biological processes within the plant cell to bring about desirable changes through the use of bioregulators. We are concerned with the use of bioregulatory agents for biochemical manipulation of the plant--the manipulation of the genetic expression of the plant to help the plant express itself more efficiently.

In early work, a great deal of our research effort was directed toward the development of more effective bioregulators for improving the color and provitamin A content of oranges and other citrus fruits. These studies resulted in improved bioregulators that elicit more appropriate color and provitamin A response pattern in citrus. Concurrently, investigations on the mode of action of the bioregulators were initiated. Results from these studies indicated that, for example, the high lycopene inducers work mainly by derepression of the gene(s) controlling carotenoid biosynthesis and inhibition of the cyclization enzyme(s) that give rise to the cyclic carotenes.

Later we reached a stage in our overall developmental work where more emphasis could be placed on studying the factors that are essential for putting the bioregulators into commercial use. Studies on the parameters of treatment under both preharvest and postharvest conditions were undertaken.

Work supported in part by the California Citrus Advisory Board and the Florida Citrus Commission.

Preharvest treatment studies were extended to the regreening problem in Valencia oranges. Appropriate bioregulators were developed for investigations on the inhibition of the regreening process in Valencias. Initial results are encouraging.

Recently we have broadened the scope of our research efforts on bioregulators to include studies on increasing quality attributes in citrus fruits in addition to those of color and provitamin A. Initial efforts in this phase were directed toward the bioinduction of antifungal activity in lemons and other citrus fruits to cause an extension in their storage life.

A brief discussion of the current status of the research on bioregulators in citrus will be given.

RECENT STUDIES ON DIHYDROCHALCONES AND TASTE MODIFIERS

Bruno Gentili and Robert M. Horowitz
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

The effect of varying the sugar moiety on the taste of sweet dihydrochalcone glycosides is an area of much interest, and one in which progress has been slow because of difficulties in synthesis. Although the sugar moiety is not required for sweetness in dihydrochalcones, its presence has a profound effect on taste quality, taste intensity and solubility. Among other compounds prepared during the past year were the 3"-O-methyl ether and the 6"-O-methyl ether of hesperetin dihydrochalcone 4'-β-D-glucoside (HDG) (the methyl ether groups are attached to the glucose part of the molecule). The 3"-O-methyl ether of HDG was found to be somewhat less sweet than HDG but the 6"-O-methyl ether was considerably sweeter. These data will be compared with some obtained earlier for other sugar-modified derivatives.

Neodiosmin, the flavone analog of neohesperidin, is a taste modifier that appears capable of partially suppressing the bitterness of limonin, naringin and a variety of other bitter substances. The synthesis of neodiosmin from neohesperidin will be discussed. The application of high-pressure liquid chromatography to the analysis of neodiosmin and neohesperidin dihydrochalcone will be described.

RECENT RESEARCH DEVELOPMENTS ON GRAPEFRUIT PRODUCTS

Robert E. Berry
Citrus and Subtropical Products Laboratory
Winter Haven, Florida

Several recent research findings concerning grapefruit juice and oil composition, sections and syrup from grapefruit serum are reviewed. Recent composition studies have indicated the presence of four coumarins, four psoralens and three methoxyflavones which have been isolated and identified from grapefruit oil. Five of these compounds have not been reported as constituents of grapefruit oil. Some quantitative data and flavor threshold values have been determined. Several appear to be present in single-strength and concentrated grapefruit juice in sufficient quantity to influence flavor of the juice. Several coumarins have been reported to impart an astringency and "greenish" or immature flavor to grapefruit juice.

In a further study of the major volatile components of cold-pressed white Florida grapefruit oil, 24 individual compounds were quantitatively analysed by gas chromatography. Using corrected weight percentages, calculated from response factors and percentages of non-volatiles in the whole oil, 19 of these components were determined quantitatively. Of the reported compounds, nine had been previously reported quantitatively, in the literature, and amounts of these were verified in the current study. In addition, two major esters, octyl and neryl acetates, are reported quantitatively for the first time as constituents of cold-pressed white Florida grapefruit oil.

Two additional studies have been carried out on enzymatic treatment of grapefruit for sectioning, and for preparation of a color stable syrup from juice serum. In developing an improved treatment to avoid the conventional steam and hot alkali treatment for preparing grapefruit for sectioning, vacuum infusion of pectinase into the peeled and unpeeled fruit was studied. Ten minutes infusion of pectinase solution caused a loosening of the peel and outer segment membrane, and albedo could be easily removed by brushing lightly. Segments which were excised from the core and adhering membrane, released easily, retained their form and were less bitter than segments from untreated fruit. Naringin and pectin substances were expressed from the peel and could conceivably be recovered in a commercial process. This system shows potential for reducing waste disposal problems, as well as quality improvement of grapefruit sections.

Grapefruit juice serum was debittered enzymatically and filtered through several different adsorbants and/or ion exchange resins. After centrifugation, the juice serum was concentrated to about 75° Brix on a vacuum rotovap and tested for color and

microbial stability during storage at elevated temperatures. While some such syrups tended to darken with time at 85°F (29.4°C), crystal clear initial syrups prepared from sera which had been filtered through both cation and anion exchange resins showed a very high color stability. These syrups did not darken after 90 days or longer at 85°F (29.4°C) and show a potential for use as sweeteners in formulated citrus products. They might be used as a source of natural sweetening and thus make it possible to increase °Brix or Brix/acid without the addition of sugar or artificial sweeteners.

STUDIES ON SOLUBILIZATION AND CRYSTALLIZATION OF HESPERIDIN IN LEMON PEEL AND LEAF TISSUE*

Raymond D. Bennett and Ronald E. Schuster
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

Previous work has suggested that the crystallization of hesperidin during extraction of lemon juice from fruit can best be explained by the reaction of a soluble complex of hesperidin in the albedo with another complexing agent, i.e., hesperidin-X + Y ⟶ hesperidin + X-Y. This would require that the two factors be in separate compartments in the intact fruit and then come in contact when cell membranes were disprupted. A polysaccharide fraction having hesperidin-solubilizing activity was isolated from an aqueous extract of albedo. A solution of hesperidin in this material was then used to test other fractions for Y-factor activity. Initially no such activity was detected. To investigate the possibility that the Y-factor was destroyed by enzymes during the isolation procedure, albedo tissue was homogenized in acetone-water (3:2) and then boiled to destroy any enzyme activity. Under these conditions Y-factor activity was found in a crude pectin fraction. Treatment of this fraction with cupric acetate precipitated the active material, while leaving most of the pectin in solution. Disc gel electrophoresis showed two major components, one of which remained at the origin. Column chromatography on DEAE cellulose separated the two, and the one which was electrophoretically mobile had Y-factor activity. This material analyzed for 94% galacturonic acid, and on acid hydrolysis no neutral sugars were obtained. Thus, it appears to be a pectin. The infrared spectrum indicated a high degree of methylation, as did its elution behavior on DEAE cellulose.

The polysaccharide fraction referred to previously which had X-factor activity was obtained only in low yield, and efforts to purify it were unsuccessful. Lemon leaves also contain soluble hesperidin, but it does not crystallize when the tissue is disrupted, which indicates that the Y-factor may not be present. This suggested that isolation and purification of the X-factor from leaves might be simpler than from albedo. When the same fractionation scheme used to isolate the albedo material was applied to leaves, hesperidin-solubilizing activity was found in a different fraction than that found in albedo. This material was chromatographed on a Sephacryl column and a partical separation of neutral and acidic polysaccharides was achieved. However,

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about 90% of the material applied to the column was not eluted, apparently because of its low molecular weight, and the polysaccharides had relatively low solubilizing activity. The latter were further separated by polyamide chromatography, but this resulted in almost complete loss of activty. These findings suggest that the solubilizing factor in the leaves is of relatively low molecular weight, but it tends to associate with the high molecular weight polysaccharides. When leaves were extracted with aqueous acetone, the soluble material, which should be mainly of low molecular weight, was found to have hesperidin-solubilizing activity. The active material passed through an ultrafiltration membrane with a molecular weight cutoff of 10,000. Efforts to isolate and characterize this material are in progress, and the hesperidin-solubilizing factor in the albedo will be reinvestigated in the light of these findings.

The phenomena of hesperidin solubilization and crystallization have also been studied at the cellular level. Living cells have been isolated from lemon albedo by treatment with a pectinase enzyme. Further treatment with a cellulase enzyme removes the cell walls and produces spherical bodies called protoplasts, which are still alive and are surrounded by the cell membrane. Bursting of this membrane, either spontaneous or by lowering the osmotic pressure of the medium, releases the cell vacuole. Usually hesperidin crystals were only observed within cells when the cell membrane had broken and the cellular contents were mixed. Likewise, protoplasts did not contain crystals, except for a few cases in which large crystal formations were observed attached to either the inner or outer surface of the vacuole membrane. However, in isolated vacuoles from some lemons, hesperidin rapidly crystallized. These crystals appeared to occupy several percent of the vacuole volume, indicating a hesperidin concentration in the vacuole of more than 1000 times its water solubility.

LIMONOIDS IN CITRUS SEEDS: COMPOSITION, ACCUMULATION AND NEW LIMONOIDS

Shin Hasegawa, Raymond D. Bennett and Carl P. Verdon
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

A preharvest treatment of citrus with triethylamine derivatives appears to have a potential use in reducing the content of limonoids in the fruit. Such a treatment requires basic knowledge about the biochemistry of limonoids. Radioactive tracer work is the best approach to establish the biosynthetic pathways of limonoids in citrus, and the choice of the species to be used is very critical. Therefore, we have made a survey of the relative composition of limonoids in various citrus species. Since limonoids are present in citrus seeds in high concentration, we used the seeds for this purpose. Practically no data regarding the relative concentration of limonoids in the seeds are available.

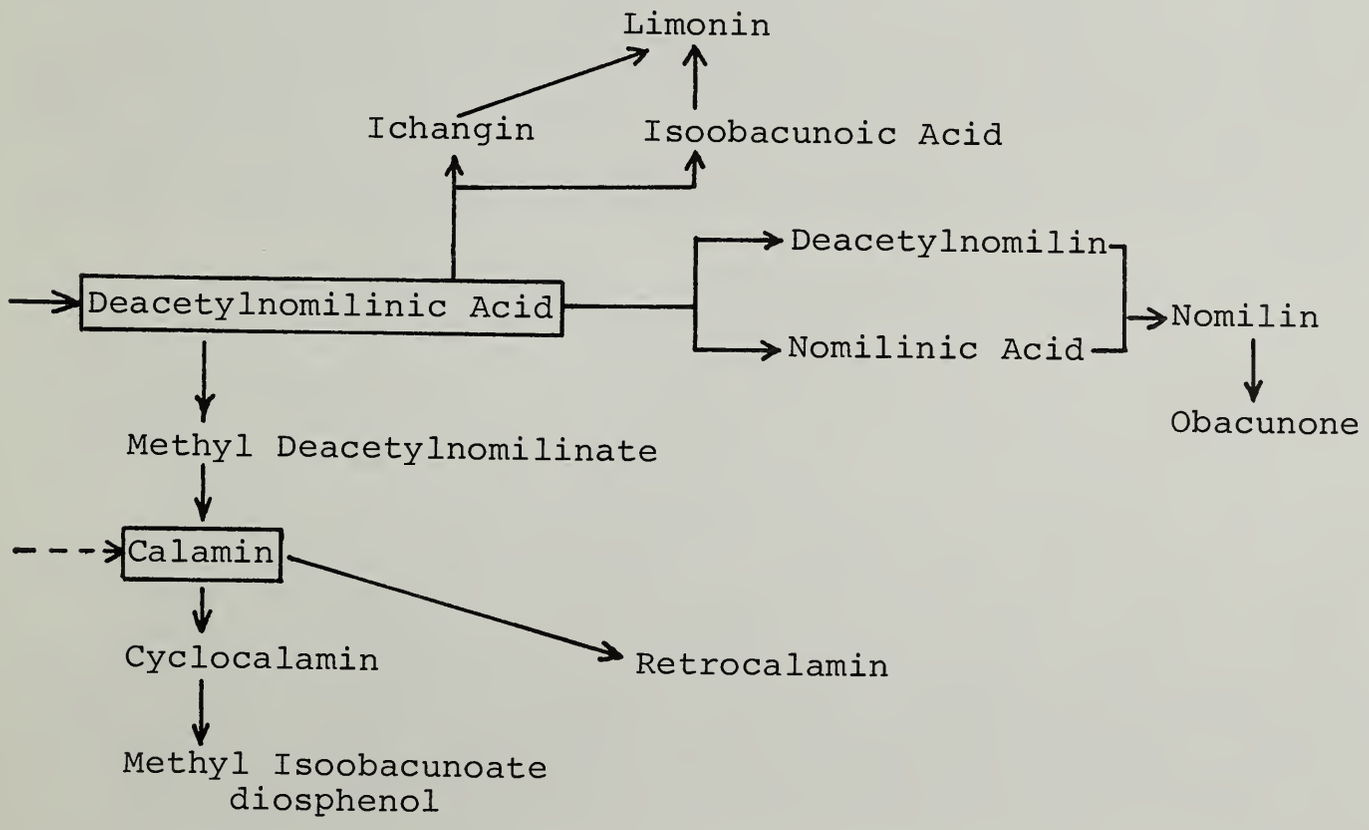
We have developed a limonoid extraction procedure. The seeds contain an enzyme, limonin D-ring lactone hydrolase, which opens the D-ring lactone of limonoids to produce water-soluble salts of the 16-carboxyl group. Therefore, we homogenize the seeds with aqueous buffer, at pH 8.5, and filter the homogenate to remove most other constituents. The filtrate is then acidified to close the lactone ring and the limonoids are extracted with chloroform. The chloroform extractions removes most water soluble constituents.

Tlc analyses showed that limonin, nomilin, deacetylnomilin and obacunone are the major limonoids in seeds of grapefruit, lemon, tangerine, navel orange and Valencia orange. Limonin was highest in all species, followed by nomilin, except for tangerine, which contained more deacetylnomilin than nomilin. Only a trace amount of obacunone was present in tangerine, navel orange and Valencia orange seeds. Grapefruit seeds contained a relatively high amount of obacunone, but they were low in deacetylnomilin. Lemon seeds, on the other hand, contained all of these limonoids in relatively high concentration.

We found several new limonoids in seeds of Calamondin (Citrus reticulata cv. 'Austera' X Fortunella sp.). We have isolated five of them, and their structures have been determined. They are named calamin, cyclocalamin, retrocalamin, methyl isobacunoate diosphenol and methyl deacetylnomilinate. Kumquat (Fortunella margarita) seeds also contained a very high concentration of calamin, four times as much as limonin, indicating that the new limonoids present in Calamondin seeds are inherited from Fortunella.

Quantitative analyses of limonin and nomilin, and their open D-ring precursors in leaves, fruit tissues and seeds during the growth of lemon suggested that limonoids are not synthesized in seeds, but rather translocated from the fruit tissue. Incubation of immature and mature lemon seeds with ^{14}C labelled acetate and mevalonate showed that labelled substrates were not incorporated into limonoid molecules. Radioactive tracer work showed that citrus trees are capable of translocating limonoids from leaves to seeds.

Based on the naturally occurring limonoids in citrus, possible biosynthetic pathways of limonoids in citrus are proposed. Deacetylnomilinic acid is most likely the key intermediate precursor of limonoid biosynthesis. Limonin could be synthesized from deacetylnomilinic acid through two pathways: one via ichangin and the other via isoobacunoic acid. Nomilin and obacunone may be synthesized from deacetylnomilinic acid via deacetylnomilin and via nomilinic acid. Calamondin limonoids also might be synthesized from deacetylnomilinic acid via methyl deacetylnomilinate. However, it should be noted that this group differs in their structures from other naturally occurring citrus limonoids in that the carboxyl group at C-3 is methylated and also the B-ring is oxygenated at C-6. Because of these unique differences in their structures, the calamin group might be synthesized through a different pathway.



Proposed Scheme for Biosynthetic Pathways of Limonoids in Citrus

SEASONAL VARIATION OF BITTERNESS COMPONENTS AND OTHER QUALITY FACTORS IN THE TEXAS COMMERCIAL CITRUS PACK

Roger F. Albach, George H. Redman, Robert R. Cruse
and Bruce J. Lime
Foods Crops Utilization Research Laboratory
Weslaco, Texas

Excessive bitterness due to limonin in orange juice or limonin and/or naringin in grapefruit juice lowers acceptability and sales. Little quantitative data are available on these components of the commercial citrus pack. Although means are available to ameliorate excessive bitterness by blending, adjustment of processing parameters, or by the newer methods of removing the bitter component by physical or enzymatic means; the appropriate application of these methods requires a knowledge of the relative magnitude of the components in the products.

The three principal citrus processing plants in the south Texas citrus belt provided triplicate samples of finished product direct from their processing lines. Samples were obtained at both one-fourth and three-fourths of the way through the days run. Sampling was repeated at approximately 3-week intervals from early November to late June where production schedules permitted. All samples were frozen as received and analyzed together at the end of the season. Concentrate samples were reconstituted to 12° Brix for orange and 10.5° Brix for grapefruit. Quality parameters determined were limonin, Davis naringin value, pulp, pH, acid, °Brix, oil, ascorbic acid, and color. Two samples from each time and date of collection were analyzed separately, and all analytical determinations were run twice on each sample.

Pulp content of orange juices differed significantly ($p=0.05$) and consistently between plants packing single-strength juices. In the single-strength samples, obtained at different times of the same day from the same plant, pulp content was not consistently associated with limonin content. Plant B orange juice which consistently contained more pulp than plant A orange juice for similar dates, contained more limonin until mid-February; afterward, limonin content was lower in juice from plant B than that from plant A. These results suggest that pulp content alone is not the principal factor determining limonin content of processed juice. With single-strength grapefruit juice, we again found no consistant correlation between relative pulp content and limonin content. However, elevated Davis-test naringin values in single-strength grapefruit juice from plant B did correlate with the higher pulp content through February, after which no significant difference in pulp content was observed.

Pulp content was lower in reconstituted orange concentrate than in the single-strength products, yet limonin content was mostly higher in the reconstituted juice. Limonin concentration decreased through the season, in agreement with findings of other workers. By December for orange and March for grapefruit, no juice contained more than the nominal taste threshold level of limonin, 6 ppm.

In grapefruit juices, naringin Davis-test values trended upward from 600-750 ppm in November to 750-800 ppm during the late season.

In orange juice, ascorbic acid content remained fairly stable at 40-50 mg/100ml until late April, when it began declining. In grapefruit juice, ascorbic acid remained near 30 mg/100ml before declining also, starting in late April.

Acid, pH, ^{o}Brix, oil content, and color showed typical seasonal trends.

None of the orange juice products contained sufficient limonin, even during the early season, to constitute a serious problem. Early season orange juice could best be utilized in blends with grapefruit or late-season orange juices. In early season grapefruit juice products, up to one-fourth of the bitterness could be due to limonin; and they would be improved if blended with less bitter juice.

EFFECT OF LOW STORAGE TEMPERATURES AND POSTHARVEST FUNGICIDES ON GROWTH OF *GEOTRICHUM CANDIDUM* IN CITRUS FRUIT

Laurie G. Houck and John W. Snider
Market Quality and Transportation Research
Fresno, California
and
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

Sour rot is sometimes a severe problem of lemons shipped from the California-Arizona citrus area in warm summer months. Sour rot is one of the most unpleasant deteriorations of citrus fruit because of its putrid, sour odor, which is especially attractive to drosophila fruit flies. These insects lay their eggs in the decaying fruit and help spread the causal fungus to noninfected fruit. Sour rot is primarily a disease of ripe or older fruit held for long periods in storage or in transit, although fruit occasionally may be affected before harvest. The pathogen, *Geotrichum candidum* Lk. ex Pers., is widely distributed in soils and causes postharvest decays of several crops. Citrus fruits may become contaminated with *Geotrichum* spores in the orchard, and infection usually occurs through deep puncture wounds, cracks, or other defects. Decay spreads rapidly by contact from infected fruit to healthy fruit. Sour rot is controlled by avoiding injuries and holding the fruit at cool temperatures. The optimum temperature for growth of *G. candidum* is 25-27°C (77-80°F); little growth occurs below 10°C (50°F). Postharvest fungicides approved for use on citrus fruit for control of other decays are mostly ineffective for control of this decay.

Research on sour rot by the USDA has emphasized practical control measures. Cooperative tests with Sunkist Growers' Field Laboratory determined that sour rot was significantly reduced by holding lemons at 10°C (50°F) rather than 15°C (59°F). Chilling injury, reported to affect lemons held below 12-13°C (53.6-55.4°F), did not develop in our test fruit held for 9 weeks of storage. Fungicides and storage temperatures were extensively evaluated in *in vitro* tests with *G. candidum* grown on potato dextrose agar. Growth of the fungus was limited more by temperature than by approved postharvest citrus fungicides, Na *o*-phenyphenate, biphenyl, thiabendazole, benomyl, *sec*-butylamine, soda ash, or borax-boric acid incorporated into the agar. Imazalil, a candidate fungicide, also was ineffective. Growth responses of these organisms to fungicides at various concentrations and at several temperatures are reported. A synergestic effect between fungicides and low temperature was not observed.

AUTOMATION OF THE MICROBIOLOGICAL ASSAY METHOD FOR ORANGE JUICE CONTENT

Carl E. Vandercook and Dora C. Smolensky
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

Often it is important to be able to determine whether a particular orange juice sample is pure and likewise to determine the amount of juice in an orange juice beverage. Last year we reported on a microbiological assay which could help determine orange juice authenticity and content. The method was based on the growth of Lactobacillus plantarum in a diluted orange juice system. The amount of bacterial growth was proportional to the orange juice content and was measured turbidimetrically. This report discusses the partial automation of the assay which has resulted in a substantial savings in time.

In the assay, the growth of L. plantarum produces lactic acid. This acid formation causes a slow drop in pH of the assay mixture in spite of the buffered solution. The change in pH is a function of juice concentration along with other factors. These other factors, which include inoculum concentration, buffer strength, incubation time and temperature, can be held relatively constant. The remaining discrepancies can be further reduced by including a standard medium in each assay set and expressing the pH values of the samples relative to the standard. The assay procedure is to monitor the pH over a time interval and express it as the rate of change. The juice concentration is proportional to this rate of pH change. Details of the method and equipment will be discussed.

The total assay time has been greatly reduced as well as the actual "bench time" spent in sample preparation, inoculation and turbidity reading. The inoculation and final read-out have been completely automated. Incubation time has been reduced from 30 hours to 5 hours. As an added bonus, the sensitivity has been improved about 10-fold which should enable the assay of orange juice beverages with as little as 2% juice.

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263 South Chester Avenue, Pasadena, California 91106

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SOUTHERN REGION

Citrus and Subtropical Products Laboratory
P.O. Box 1909
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Food Crops Utilization Research Laboratory
P.O. Box 388, Weslaco, Texas 78596

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