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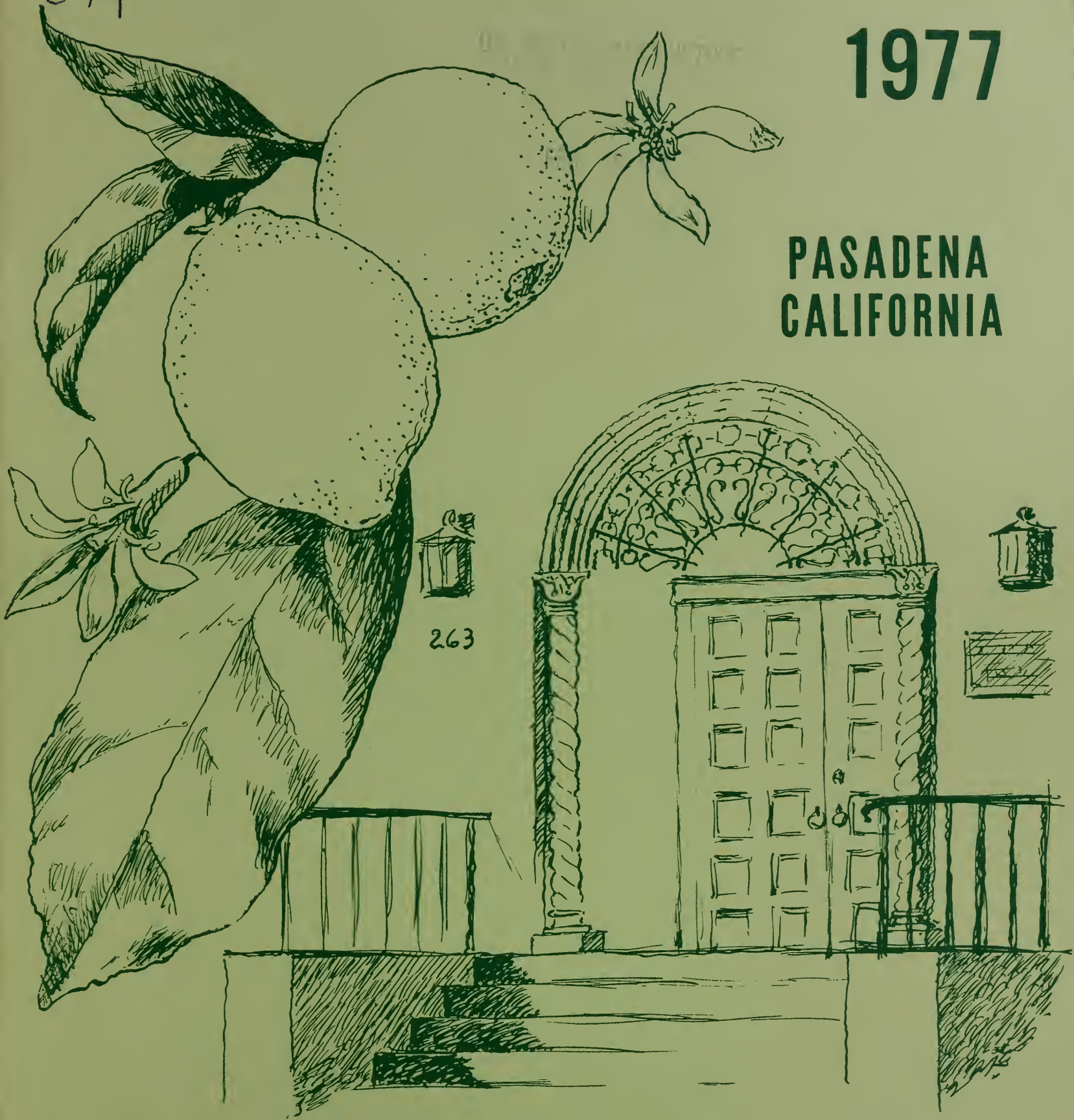

CITRUS RESEARCH CONFERENCE

CITRUS RESEARCH CONFERENCE

November 30, 1977

Pasadena, California

ABSTRACTS OF PAPERS

Sponsored By:

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

Agricultural Research Service
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 the Agricultural Research Service, 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

Western Regional Research Center
Berkeley, California 94710

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 30, 1977

MORNING SESSION - 9:00 A.M.

Abstract on page

WELCOMING REMARKS: Peter H. van Schaik
Acting Area Director
ARS/USDA, Fresno, California

INTRODUCTORY REMARKS: H C Cox, Deputy Administrator
Western Region
ARS/USDA, Berkeley, California

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

AFTERNOON SESSION - 1:45 P.M. Abstract on page

APPLICATION OF A MICROBIOLOGICAL ASSAY TO DETECT ADULTERATION IN RETAIL ORANGE JUICES AND DRINKS

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

The microbiological assay which was proposed last year as a possible method of detecting adulteration in orange juice has been modified and applied to eleven brands of retail frozen concentrated orange juice and orange drinks (10% juice).

The modifications include the use of a standard to help compensate for minor uncontrolled daily variations in the assay conditions. The growth in the orange juice samples is expressed relative to the growth in the standard.

Also, the sensitivity of the modified assay has been improved five-fold over that of the original version. By reducing the amount of juice necessary in the assay mixture it is now possible to assay orange drinks.

The increased sensitivity comes from supplementing the assay mixture with the growth limiting nutrients for *Lactobacillus plantarum* in orange juice. The first and second groups of growth limiting nutrients were determined to be the amino acids leucine, isoleucine, valine and methionine and the mineral manganese, respectively. The assay mixture was supplemented with these groups of nutrients to give a stepwise enhancement of growth over the unfortified control. The demonstrated relationship of the growth in the control assay mixture to that in the supplemented mixtures should be quite helpful in detecting substances added to circumvent the assay. This is because once the minimum requirement for a given nutrient has been met, another nutrient becomes limiting and any excess of the first produces no additional growth. For example, an adulterated product containing added amino acids would show no increased growth in the supplemented assay.

FEEDING STUDIES OF FREEZE-DRIED ORANGE PEEL PUREE AND FREEZE-DRIED ORANGE JUICE PREPARED BY SIMULATION OF THE WHOLE ORANGE PUREE PROCESS

Michael R. Gumbmann*, Daniel Gould* and Roger F. Albach**
*Toxicology and Biological Evaluation Research Unit
Western Regional Research Laboratory
Berkeley, California
and
**Food Crops Utilization Research Unit
Weslaco, Texas

The nutritional value of citrus whole-fruit puree is largely unknown; due both to its limited traditional consumption among humans, and the relatively meager efforts to characterize its chemical composition.

Cattle feeding experiments have established that long-term consumption of either raw or dehydrated whole oranges or grapefruit was absolutely harmless to the animals. The studies reported here are the first on record to deal with non-ruminant animals.

Although there is no historical or scientific evidence to suggest that consumption of whole citrus or citrus peel may have detrimental effects, there is presumptive evidence in the literature which suggests that further research is needed to establish the effect of citrus peel and its components on the health of the consumer. The presumptive evidence consists mainly of various citations in the literature to known components of citrus peel which are biologically active and potentially antinutritive in character. It is the function of the research reported here to access the merits of these presumptions by test feeding freeze-dried orange peel puree and freeze-dried orange juice.

On November 29, 1974,'Hamlin' oranges were harvested from a grove at the Texas Agricultural Experiment Station, Weslaco, Texas. The grove received documented care similar to that used in commercial orchards. At the Food Crops Utilization Research Laboratory whole oranges were water blanched as in the initial step of the whole orange puree process. After juice extraction, the peel and pulp underwent the remaining steps in the puree process of comminution, homogenization, and pasteurization. The juice was also pasteurized. Both the puree and juice were freeze-dried. At the Western Regional Research Center the freeze-dried products were fed separately at 30% of total diet to weinling rats for 45 and 90 days prior to necropsy. Body weights of the test and control animals were recorded at two-week intervals. Blood samples were collected at the time of necorpsy for hematology and enzyme and chemical analyses. Organ weights, and gross pathological and histological examinations were made. The only

adverse effect that was clearly evident at this high percentage of total diet was decreased body weight in the group fed the 30% freeze-dried orange peel. Some liver enlargement observed in this same group is most likely the result of microsomal stimulation which is not necessarily a toxic effect. Histological examination of various organs failed to disclose any lesions which were associated with any of the three dietary groups.

FORMATION OF HESPERIDIN CRYSTALS IN LEMON JUICE CLOUD*

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

Microcrystalline needles of hesperidin are an important part of lemon juice cloud. Most of the hesperidin is derived from the fruit albedo, in which it is present in a soluble form. When the albedo tissue is disrupted during juice extraction, hesperidin, which is highly insoluble in water, crystallizes rapidly. This process could be explained by conversion of a soluble precursor, such as a chalcone, to hesperidin, either spontaneously or by the action of an enzyme. However, no such precursor could be isolated after extraction of lemon albedo by various methods, including conditions under which the chalcone is stable. Also, crystallization of hesperidin was not prevented by treatments designed to destroy enzyme activity. These observations indicate that hesperidin is present intact in the albedo, kept in solution as a complex with a solubilizing factor. This complex must then be broken up during juice extraction. Since destruction of the solubilizing factor by an enzyme appears to be ruled out, the most reasonable explanation would be dissociation of the complex by a competing factor, i.e., hesperidin-X + Y → hesperidin + X-Y. The complex and the Y-factor would be in separate compartments in the albedo tissue but would come in contact when cell membranes were broken. A model system of this type was prepared in which X was aluminum ion and Y was pectic acid. A soluble hesperidin complex was formed, and hesperidin crystallized when pectic acid was added. However, lemon albedo contains too little aluminum for this to be the natural process, and the metal ions present in high concentrations do not form soluble complexes with hesperidin.

Attempts to isolate the soluble complex from albedo by various extraction methods were unsuccessful. In all cases the complex was broken up and hesperidin crystallized. An aqueous extract of albedo showed no hesperidin-solubilizing activity. However, when the polysaccharides of this extract were fractionated by solvent precipitation and treatment with cupric acetate, an active material was obtained. On paper electrophoresis a single major component was observed, and a colorimetric assay showed a galacturonic acid content of 60%. The neutral sugars obtained by acid hydrolysis were arabinose, xylose, and glucose, in approximately equal amounts. This polysaccharide enabled a solution of

*Work supported in part by the Citrus Products Technical Committee.

hesperidin to be made more than ten times as concentrated as its normal water solubility. When such a solution was treated with a pectin fraction obtained from the same extract as the active material, the complex was dissociated and the hesperidin crystallized. This suggests that the Y-factor referred to previously is a pectin. Progress in isolating and characterizing this substance will be discussed.

Lemon leaves also contain hesperidin in soluble form, but when leaf tissue is disrupted no crystalline hesperidin is obtained. Apparently the Y-factor of the albedo is not present in leaves. It remains to be determined if the solubilizing factor in the leaves is the same as that in the albedo.

EXPERIMENTS ON THE METABOLIC FATE OF DIHYDROCHALCONES AND OTHER FLAVONOIDS

R. M. Horowitz, Bruno Gentili and Shin Hasegawa
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

The work reported a year ago on the metabolic fate of neohesperidin and neohesperidin dihydrochalcone has been continued. In the rat the major urinary metabolite produced from these compounds is evidently a carboxylic acid, since it appears to undergo esterification in methanol-hydrochloric acid. Thus far, attempts to apply high-pressure liquid chromatography to the separation of metabolites in urine have not been successful.

The microbial metabolism of neohesperidin dihydrochalcone and related flavonoids is being examined, as it might provide a useful model of mammalian metabolism. The aglycone hesperetin dihydrochalcone was cleaved by an unidentified soil bacterium to dihydroisoferulic acid and phloroglucinol. The ratio of these products was about 10:1, from which we conclude that the phloroglucinol becomes the primary source of carbon for the organism. This was substantiated to some extent by showing that the organism grows (slowly) on phloroglucinol alone, thereby forming a new crystalline substance whose structure is being studied. The organism acted reluctantly on neohesperidin dihydrochalcone, probably because it cannot hydrolyze the sugars. Most of the starting material remained unchanged but a small amount of dihydroisoferulic acid was formed and there was chromatographic evidence for a trace of isovanillic acid. When hesperetin was used as substrate it was metabolized to only a small extent; we obtained a very small amount of a pure metabolite which, in its spectral properties, was reminiscent of a chalcone. These findings will be compared with earlier results reported in the literature.

A brief summary will be given of the current status of dihydrochalcone sweeteners with regard to toxicology, applications and acceptability.

EXTENSION OF WORK ON BIOREGULATORS IN CITRUS*

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

In the past year 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. This extension reflects, in part, our continuing interest in the development of bioregulators possessing multi-effects. We have initiated investigations on the influence of bioregulators on the storability of citrus fruits. Fungal decay adversely affects the storage life of citrus fruits and creates major problems particularly in those fruits shipped to export markets. A number of reports have appeared in the literature associating terpenoid compounds with antimicrobial activity. So, it became of interest to see whether the induction of antimicrobial property in citrus fruits is possible--whether bioregulators can elicit this desirable response in citrus fruits. These investigations are being conducted in collaboration with Drs. A. Dawson and P. Harding of Sunkist. Lemons were selected for these preliminary studies because they are more readily susceptible to fungal decay than, for example, oranges. Initial results suggest that it might be possible to induce antimicrobial activity in citrus through the use of bioregulators. Tests run by Dr. B. Davé at Decco showed that the bioregulators used in these studies do not in themselves possess an antimicrobial property. No antimicrobial activity was observed in postharvest treated lemons. However, resistance to *Penicillium* decay was observed in preharvest treated fruits. Apparently the *Penicillium* resistant activity is induced only under preharvest conditions. It remains to be determined whether the leaves are involved in the formation of the fungal resistant activity. It also remains to be seen whether this approach will be effective against a broad spectrum of fungi involved in citrus storage problems. Not all of the bioregulators appear to cause the induction of antimicrobial activity in lemons; only those with certain structural features appear to be effective. Detailed structure-activity relationship remains to be established.

*Color work supported in part by the California Citrus Advisory Board and the Florida Citrus Commission.

In our overall developmental work on enhancement of color and provitamin A in oranges and other citrus fruits, we are continuing to place increased emphasis on studying the factors that are necessary for putting the bioregulators into commercial use. Studies on parameters of treatment under both preharvest and postharvest conditions have continued. Correlation of timing of preharvest application of the bioregulators to fruit maturity and improved formulation were studied in greater detail relative to more uniform color response in oranges.

CURRENT STUDIES ON PREHARVEST PREVENTION OF REGREENING IN VALENCIA ORANGES*

Wan-Jean Hsu, Steven D. Lee, Charles DeBenedict
Stephen M. Poling and Henry Yokoyama
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

The phenomenon of reappearance of the green pigment chlorophyll in mature Valencia oranges [*Citrus sinensis* (L.) Osbeck] is known as regreening; and it is a problem in California and Florida. Last year we briefly reported preliminary yet encouraging results about preharvest prevention of regreening in Valencia oranges. Six bioregulators examined in the field test appeared to interfere with the regreening process and showed promise as potential inhibitors.

Further studies on the preharvest prevention of regreening were conducted this year in Riverside and Ivanhoe (San Joaquin Valley), California. Twelve bioregulators including last year's potential ones were tested. The bioregulators were applied by carefully spraying the 20-30 fruits on a section of the tree; an equal number of fruits on adjacent branches were left untreated and used as controls. The fruits were treated on June 3, and again on July 6, 1977. Of the twelve bioregulators examined in Riverside, California, seven appear to interfere with the regreening process confirming the results obtained last year. In four cases the inhibition of regreening also appeared to be accompanied by enhancement of orange coloration of the rind.

During the three month observation period (June 3, to Aug. 29, 1977), the regreening process progresses. The chlorophyll content in the flavedo increases and the total carotenoid content decreases in all the fruit. At the end of the first and second months the flavedo of the treated fruit contained higher amounts of total xanthophyll as compared with that of the control fruit, and much lower total chlorophyll content than that of the control fruit. However, at the end of the third month, the treated fruit turned much greener than before although they still remained less green than the control fruit. This showed that the duration of the action of these bioregulators is about one month. Repeat of monthly treatment during the summer months seems necessary. Further field tests on Valencia oranges in midsummer for prevention of regreening will be conducted next season. Efforts will also be continued toward the development of more effective bioregulators to prevent regreening and at the same time enhance the orange coloration of Valencia oranges, that is, toward the development of multieffect bioregulators.

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

COLOR ENHANCEMENT OF CITRUS BY THE CHEMICAL INDUCTION OF POLY-*CIS*-CAROTENOIDS*

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In past years, we have reported the enhancement of color in citrus by various classes of tertiary amines. These amines work mainly by causing lycopene accumulation in the flavedo by the derepression of the gene(s) controlling carotenoid biosynthesis and the inhibition of the cyclization enzyme(s) that gives rise to the cyclic carotenes. Postharvest studies have shown that although the initial affect of these tertiary amines is lycopene accumulation, the aliphatic esters of 2-diethylaminoethanol, such as 2-diethylaminoethyl hexanoate, caused the eventual accumulation of β-carotene as the major carotene pigment. Although the aliphatic esters cause rapid lycopene accumulation during the first few days, they are rapidly hydrolysed and become inactive. There is no further stimulation but also no more inhibition of the cyclase(s) and the accumulated lycopene is converted to the provitamin A β-carotene. On the other hand, the non-ester tertiary amines, such as 2-(*p*-methylphenoxy)-triethylamine, cause a very large lycopene accumulation in the fruit because they remain active, continually stimulating biosynthesis of carotenoids but also inhibiting the cyclase(s). The esters of substituted benzoic acids, such as 2-diethylaminoethyl *p*-bromobenzoate, cause a response intermediate between the two previous classes. Because they are more slowly hydrolysed than the aliphatic esters, lycopene is accumulated as the major pigment although there is a significant increase in β-carotene.

Initial postharvest studies of the lycopene inducers showed that while it was possible to enhance the color of the peel of fruit destined for the fresh fruit market, there was a tendency for the fruit to become unnaturally red. Further studies using preharvest application of the bioregulators seem to indicate that this problem can be overcome and the lycopene inducers may be useful for fruit destined for the fresh fruit market. The effect of the lycopene inducers in the endocarp both pre- and postharvest, on the other hand, seems to be fundamentally different. Lycopene is not accumulated, but there is a significant increase in the orange xanthophyll pigments. The lycopene inducers therefore show very good promise for use as color enhancers for fruit destined to be juiced.

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

Although the lycopene inducers show promise as color enhancers for fruit destined for the fresh fruit market, a more ideal bioregulator would be one which only caused the fruit to accumulate orange pigments thus avoiding the problem of overly red fruit. We have developed a new class of bioregulators that cause the fruit to develop only an orange color and never become red. This new class is based on substituted dibenzylamines or benzylfurfurylamines. The most active of these compounds, i.e. *p*-bromobenzylfurfurylamine, causes lemons and grapefruit to develop the same orange color as good colored oranges. This color enhancement is caused by the accumulation of poly-*cis*-carotenoids. Normally most plants synthesize and accumulate only the all *trans* carotenoids. The *trans* configuration is the most stable isomer. Chemical isomerization with iodine of poly-*cis*-carotenoids will result in an equilibrium mixture that is predominately the all *trans* form. Whether the plant accumulates *trans* or *cis*-carotenoids seems to be genetically controlled. The tangerine tomato, which is orange, has a double recessive gene which causes it to accumulate poly-*cis*-lycopene (prolycopene) as the major pigment. The bioregulators in this new class cause citrus to accumulate prolycopene, proneurosporene, pro-γ-carotene and prorubixanthin, the poly-*cis* forms of the corresponding carotenoids, as well as *cis*-lycopenes with fewer *cis* bonds than prolycopene. Although prolycopene is the major pigment, it does not predominate over the other procarotenoids to the same degree as lycopene predominates over the other carotenes in fruit treated with lycopene inducers. We believe that this new class also acts at the genetic level by derepressing a gene that is usually not expressed or only weakly so. These new bioregulators also differ from the lycopene inducers in that they do not seem to inhibit the cyclase(s).

The reason the accumulation of prolycopene causes the fruit to become orange and not red is because the conversion of five of the unhindered *trans* double bonds to *cis* double bonds shifts the absorption maximum to lower wavelengths. Converting from lycopene to prolycopene shifts the maximum from 469 nm to 434 nm. There are similar shifts to lower wavelengths with the other procarotenoids. The xanthophylls that give oranges their orange color absorb around 435 nm so that the change from all *trans* to poly-*cis* causes the induced carotenoids to absorb in the same region and give the same color. The other significant spectral change that occurs when changing from all *trans* to poly-*cis* is a lowering of the extinction coefficient. This means that more procarotenoids must be accumulated than *trans* carotenoids to give the same color intensity. It may be necessary to develop more active bioregulators of this new class to get the desired results.

Thus we have shown that the possibility exists of enhancing the color of citrus by the induction of poly-*cis*-carotenoids with this new class of cynthetic bioregulators. The advantage of these bioregulators over lycopene inducers is that the fruit only develops an orange color and never becomes red. This would be most advantageous for fruit destined for the fresh fruit market.

RESEARCH ON USE OF ENZYMES IN CITRUS PROCESSING

Joseph H. Bruemmer
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Winter Haven, Florida

Grapefruit salad sections are prepared commercially by steam and lye peeling followed by excision of the segments from their membranes. This procedure removes the albedo and segment membranes which contain the bitter substance, naringin. The peel can also be removed by enzyme treatment. Vacuum infusion of pectinase into prescored fruit softens the albedo. After pectinase treatment the peel is removed easily and the segments separate readily from the membrane and seeds.

Another enzyme, naringinase, can be used to debitter the albedo and segment membrane so that they can be consumed with the segments. Vacuum infusion of naringinase into flavedo-shaved grapefruit hydrolyzes the naringin to less bitter and non-bitter products. When flavor substances, sweeteners, and nutrients are incorporated into the infusate, the albedo becomes a nutritious and tasteful adjuvant to the grapefruit segments.

Pectinase can be used to prepare high Brix orange juice syrups. Orange serum prepared by clarifying juice with polygalacturonic acid (PGA) forms a gel when concentrated to a syrup. Pectinase treatment of the serum depolymerizes the clarifying agent, PGA, and the pectic substances native to the juice. The treated serum can be concentrated to a fluid 85^0 Brix syrup that is microbially stable at ambient temperatures. Chemical stability of the syrup can be improved by removing the amino acids and other cations with ion exchange resins. The high Brix syrups can be used to prepare low pulp juice concentrates that are microbially stable at ambient temperature.

PROGRESS IN RESISTIVE PULSE PARTICLE SIZE ANALYSIS

A. W. Venolia
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The optical properties of aqueous suspensions are ordinarily strongly influenced by such factors as refractive index and particle size. For those who are interested in complex suspensions of the kind obtained by disrupting living cells, the practical definition of what constitutes a particle can be difficult. An example is provided by an organelle whose substructure is such that it contains more or less discrete light scattering entities within itself. Another type of particle displaying such character might consist of an optically inhomogeneous fragment of cell wall. For the time being it seems appropriate to ignore such complications, but it may be well to bear in mind that particle substructure could have practical consequences.

The wavelength of visible light ranges from about 0.4 to 0.76 μm. If we are willing to make the somewhat reasonable assumption that a particle one wavelength in diameter will scatter light optimally, then the visual perception limits cited can be used to estimate the size of an optimal scatterer. For an aqueous suspension like lemon juice it is plausible to postulate a serum refractive index of about 1.34. On this basis we can judge that the particles in question would have diameters of roughly 0.4 μm.

While the development of the new resistive pulse instrument has emphasized particle size resolution and precision as well as ultimate speed and operating efficiency, we have not ruled out the possibility of extending the size range to the lower levels that are of greatest interest from a light scattering standpoint. Indeed, it presently appears that the new instrument will permit work below the 0.8 μm limit that was encountered earlier using the Model A, Coulter Counter.

It is often desirable to correct observed particle counts for the effect of simultaneous passage of more than one particle through the sensing aperture; this effect is generally referred to as coincidence. The coincidence correction we used in reducing data obtained with the Model A, Coulter Counter was described in 1965 by Princen and Kwolek (Rev. Sci. Instr., *36* 646). We recently replaced the Princen method with one suggested by Bader, Gordon and Brown in 1972 (Rev. Sci. Instr., *43* 1408). The older method relied upon an empirical constant to obtain corrected counts; each aperture had its own constant that was derived from the dependence of observed counts upon particle concentration. The newer method develops coincidence corrections directly from

the data to which the corrections will be applied, and it is therefore somewhat more flexible than the older method. The ability of the newer method to allow for the dependence of the correction upon the character of the particle size distribution is obtained at the cost of examining an increased number of samples from the parent suspension. However, by taking advantage of the possibility of linking the present instrument with a computer the apparent disadvantage of the newer coincidence correction can be reduced almost entirely to the time needed to perform the requisite dilutions.

Results of preliminary runs on a sample of commercial lemon juice will be discussed.

USE OF ISOLATED MESOPHYLL CELLS FOR BIOCHEMICAL STUDIES OF CITRUS*

Shin Hasegawa and J. E. Hoagland
Fruit and Vegetable Chemistry Laboratory
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During the past several years, techniques have been developed for the isolation of mesophyll cells from higher plants. The use of isolated cells constitutes a very useful experimental system for biochemical studies of cellular processes in plants. Recently, we have adopted this technique for biochemical studies of citrus.

This method offers at least two advantages. First, all cells in a suspension can be exposed to equivalent quantities of compounds added to the suspension. Second, the cells can be handled similarly to unicellular organisms, thus facilitating uniform samplings. Therefore, experiments can be carried out under strictly controlled conditions.

Major factors which need to be considered in the isolation of metabolically active cells include: (1) use of appropriate maceration enzymes; (2) use of an osmoticum such as sorbitol at levels in which cells are stable; and (3) a method for the efficient purification of cells from the mixture of undigested tissues, broken cells, organelles etc.

Considering the above factors, metabolically active cells were isolated from leaves of citrus. Young leaves were macerated in a medium containing 0.7 M sorbitol, 50 mM HEPES, 10 mM K_2SO_4 and 0.5% Macerase (Calbiochem) adjusted to pH 5.8. The cells released during the first 30 min were discarded, and those released during the next 60 min were collected. The detailed procedure will be presented.

Mesophyll cells isolated by this method were capable of photosynthesis. They were active even after storage in the dark for 6 hrs. Cells isolated from leaves of Eureka lemon had high rates of biosynthesis, converting all Na-acetate-2 ^{14}C of the incubation medium into numerous compounds which include labelled limonin. For the preparation of labelled limonin, this method was much more effective and less time-consuming than the previously reported method which used citrus trees in the field.

The catabolic capability of isolated mesophyll cells was also demonstrated. Cells isolated from leaves of bitter orange converted neo-hesperidin-2 ^{14}C into several metabolites, which were more polar than the substrate.

*Work supported in part by the Citrus Products Technical Committee.

These results showed that isolated mesophyll cells from citrus appeared to be ideal material for studying biosynthesis and biodegradation of citrus constituents as well as photosynthesis. This method will be used primarily for biochemical studies of limonoids, but it can be used also for other flavor and color constituents as well.

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Fruit and Vegetable Chemistry Laboratory
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*Reprints are available at the addresses indicated; patents are available only by purchase at 50¢ a copy from the U.S. Patent Office, Washington, D.C. 20231.

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SOUTHERN REGION

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CLARIFICATION OF CITRUS JUICES WITH POLYGALACTURONIC ACID
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