color that occurred during storage of the packs of canned grapefruit sections collected from plant A during the 1953-54 season, it was decided to obtain samples from two other plants during the following season in order to confirm such results. However, when this was done, the data obtained, as shown in Tables 2 and 3, indicated that the differences found after storage at 90°F for six months in flavor and color in the packs from plants B and C were not only slight, but quite uniform, and that there was no evidence to show that these changes could be correlated with the date of packing. Thus, other factors are perhaps more important than the packing date in causing changes in flavor, firmness, and color of canned grapefruit sections during storage.

Summary

Samples of commercial packs of canned grapefruit sections, collected from three plants throughout two packing seasons, were stored at 90°F and examined for firmness, color, and flavor at monthly intervals for a period of six months. Results indicated that date of packing affected quality after storage of the samples obtained from one plant during the 1953-54 season; however, examination of products collected from the two other plants during the 1954-55 season did not confirm this relationship, since in these instances no correlation was found between the processing date and retention of quality in the stored sections.

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Literature Cited


CITRUS VITAMIN P - II. THE ANTIBIOTIC ACTIVITY OF CITRUS BIOFLAVONOID**

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In our paper, presented at last year's Annual Meeting of the Florida State Horticultural Society, we reported the experimental and clinical data concerning the therapeutic usefulness of citrus bio-flavonoids in capillary injury. We pointed out that in various conditions such as brain hemorrhage, nose and gum bleeding, miscarriage, hemorrhagic duodenal ulcer and other diseases where capillary bleeding is present, citrus bio-flavonoids are beneficial.

In the present report, we are dealing with a question which might interest many citrus growers and producers, a question which to many may appear to be controversial and unresolved: Are citrus fruits and juices, oranges and grapefruits useful and beneficial

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in bacterial and viral infections?” The clinical studies conducted in the past with orange juice and oranges have given inconclusive results, as many clinical trials of this type often do.

Modern medical science depends in its conclusion about the therapeutic value of this or another product much more on the experimental work, which is conducted under precise and controlled conditions, than on clinical studies. Thus, as was the case of bio-flavonoids in capillary fragility, our present paper concerns mostly the experimental studies on citrus bio-flavonoids, naturally present in oranges and grapefruits, and their effect on viruses.

How do viruses differ from bacteria? Not only are they very small micro-organisms, much smaller than bacteria, but they possess, due to their smallness, the ability to penetrate easily into the cells of the mucous membranes of the mouth, throat, and intestine. These often vicious micro-organisms destroy the cells, multiply rapidly and infest the surrounding tissue. They frequently induce acute inflammation, which if grave, might lead to the patient’s death.

When virus particles invade the tissue of the human organisms, they also in the early stages of infection often penetrate the endothelial cells of the capillary wall. They destroy these cells and by doing so, they injure the capillary and cause capillary bleeding. It is called the capillary syndrome. In almost all virus infections the capillary syndrome is present, This means that the capillaries in the infected areas are injured and often destroyed, with multiple capillary bleeding evidenced. Death might occur from congested lungs, liver or other organs with partial or complete impairment of hundreds of small vessels. Many recent pathologic studies demonstrated that in almost all viral infections, injury to the small blood vessels, the capillaries, is present: in poliomyelitis (1, 2, 3); small pox (4, 5); in measles (6); encephalomyelitis (7); in primary atypical pneumonia (8); in mumps (9, 10); and in virus A influenza (11). In rabies, vascular congestion and prevascular hemorrhages are observed. In epidemic leukoencephalitis “the pathologic picture is one of congestion of arterioles and capillaries” according to Olitzky (12). This fact by itself suggests that citrus bio-flavonoids as substances restoring capillary integrity and controlling capillary bleeding might be useful in some viral infections. They might minimize the gravity of inflammation and bleeding present in serious cases of such infections.

But besides this indirect usefulness of citrus bio-flavonoids in virus infections, there is considerable experimental evidence indicating that some bio-flavonoids possess an activity against certain viruses, that they have an antibiotic property.

A number of papers were published on the antibiotic activity of certain flavonoids against bacteria. Naghski et al (13) found that quercitrin and the aglycone quercetin are active against Staphylococcus aureus; Andersen and Berry (14) against Clostridium botulinum. Bustinza and Lopez (15) reported the activity of these flavonoids against several types of bacteria. Schraufstatter and Deutsch (16) investigated several naturally occurring flavonoids and found that chalcones were more active against Staphylococcus aureus than the flavonoids were.

It was Dr. Windsor Cutting, Head of the Department of Pharmacology, Stanford University, California and his associates (17, 18, 19) who first pointed out that certain flavonoids (quercitrin) exerted a moderate prophylactic activity against some viruses (rabies and ectromelia viruses). The flavonoids were given to mice and rabbits by mouth. According to them, naringin possibly shows a mild antiviral activity. They believe that the presence of a double bond between the 2 and 3 positions probably enhances the anti-virus activity of flavonoids. Green et al (20) and Yoshimura et al (21) investigated crude and purified flavonoids from oranges, lemons and grapefruits, testing them on influenza virus PR 8 and Mengo encephalomyelitis viruses. Citrus bio-flavonoids were administered to mice either by mouth or by injections. The investigators arrived at the conclusion that certain flavonoid fractions naturally present in citrus fruit possess an activity against these two viruses, since the mortality rate of infected animals was decreased. Ascorbic acid added to citrus bio-
flavonoids did not enhance the anti-viral activity of these compounds.

Dr. Murray Sanders, Head, Department of Microbiology, University of Miami (22) tested the citrus bio-flavonoid compound, known as C.V.P., which contains the flavonoids naturally present both in orange and grapefruit against the Type II poliomyelitis virus (Lansing Type). He used 685 CFW mice, for the control and treated groups. The animals were given the flavonoid compound by mouth, for several days, before and after virus inoculation. He found a definite yet moderate prophylactic action of citrus bio-flavonoids against the virus disease. The paralysis was delayed considerably and the number of mice which survived was higher in the treated group than in the control (18% against 4%). Chamelin (23) conducted an extensive investigation with the same compound and its various fractions. He tested their activity against vaccinia virus by the chick-embryo technique. He found that certain flavonoid fractions had an activity from x 100 up to x 250. According to him, water-insoluble hesperidin shows no antiviral activity, an observation which was made previously by Cutting et al (19) and Green et al (20). Kato (24) reported that certain highly purified fractions of the citrus bio-flavonoid compound possessed an activity ranging from x 100 up to x 250 against mouse influenza virus PR 8. He tested them both in the chick-embryo and on mice inoculated with the influenza virus. He stressed the fact that while some citrus flavonoids have an antiviral activity, others have none.

In view of the evidence that certain citrus flavonoids exert an antiviral activity both in vitro in the tissue culture and the chick-embryo, and in vivo, one may conclude that this action is of a true antibiotic nature. A large number of clinical trials were conducted with bio-flavonoids in treatment of colds, influenza and upper respiratory infections. Many investigators (25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37) reported beneficial and often dramatic results from this therapy. On the other hand, a number of clinical studies resulted in negative end-results (38, 39, 40). This discrepancy between the papers published on this subject might be explained by the fact that various investigators have used different flavonoid compounds, some of which might have been inactive. The inadequate dosage of the preparation given to the patients also should be taken into consideration. As Cutting et al (18) pointed out, a large daily dosage of flavonoids should be administered (orally) to animals in order to protect them against viral infection. In a small series of influenza A virus cases, sero-diagnosed, it was demonstrated (32) that citrus bio-flavonoids should be administered every three hours, with a total dose of about 8 grams a day, in order to be therapeutically effective in human influenza.

During the last three years, the Virus Division of the Southern Bio-Research Laboratory has been involved in research concerning the activity of citrus bio-flavonoids against various viruses. Mice, rats, chicks and rabbits were used for this work. Crude and purified flavonoids naturally present in orange and grapefruit were investigated, first by the so-called chick-embryo technique and afterwards on animals. In most of our tests, we investigated the citrus bio-flavonoid compound extracted from citrus wastes of oranges and grapefruits. Thus this compound contained the flavonoids naturally present both in oranges and grapefruits. The basic problem of our investigation concerning the relationship between the chemical structure of a flavonoid and the antiviral activity is not resolved as yet. We hope that we shall be able to complete this part of our work in the near future and possibly we shall be in a position to report our findings on this subject at the Annual Meeting of your Society next year.

In our present paper, we report our findings concerning the use of citrus bio-flavonoids in two conditions: ileitis, the disease which affected President Eisenhower, and influenza.

Ileitis, also known as regional ileitis or enteritis, is a relatively rare disease. In ileitis, the small intestine, particularly the part called the ileum, becomes inflamed. The inside lining of the intestine is red-purple in color. The wall is thickened, with the lymph nodes enlarged. The blood vessels of the in-
testinal wall are congested and there is gross capillary injury. The lumen of the small intestine might become so narrow that digested food cannot pass through it. In young children, ileitis as a rule is in the much more dangerous acute form and is often fatal.

A similar disease, known in rabbits, is called mucoid enteritis and affects mostly the ileum. It kills young rabbits within a few days and is of epidemic nature. A young baby rabbit, healthy today, might be close to death in 24 hours, with loss of about 20% of his weight in one day. The cause of this disease in rabbits is not known, but it is suspected that some virus is responsible for the disease. Although we are unable, as yet, to isolate this virus, the histological investigation conducted by us strongly indicates the viral nature of this affliction (the presence of inclusion bodies during the first twenty hours of infection). The citrus bio-flavonoids were used to treat this infection. The investigation was conducted in cooperation with the Hamilton Rabbit Station, Roscoe B. Jackson Memorial Laboratories in Maine. The moment that the first signs of the disease appeared, the sick animals were placed on large doses of water-soluble citrus bio-flavonoids, orally administered, in water with sugar, for five-six days. The results of these trials as recorded by Dr. P. B. Sawin, Head of the Rabbit Station, were as follows:

154 control rabbits, affected with acute enteritis, and receiving no treatment, all died. Out of 93 rabbits treated with citrus bio-flavonoids, 75 survived. Thus the mortality rate for the control rabbits was 100% against 19.5% for the flavonoid treated group.

Influenza virus PR 8 kills mice in 12-15 days after four drops of the virus solution are inoculated intranasally. In these experiments, a highly purified water-soluble citrus bio-flavonoid compound, containing flavonoids naturally present both in oranges and grapefruits, was used. First the activity of this compound, labeled C66, was tested by the chick-embryo technique, and it was established that its activity against influenza virus was x 100. The mice were given 10 mg. of the flavonoid compound, injected subcutaneously for five consecutive days, one day before virus inoculation and for four days after. The following results were obtained: out of 230 control mice inoculated with influenza virus PR 8, 22 mice or 9.4% survived. In the treated group of 250 mice inoculated with the same virus, 82 mice or 31% survived.

Thus our experimental investigation on the influenza virus PR 8 indicates that the citrus bio-flavonoid compound, C66, gives some protection to mice against the virus. This protection was far from complete. The mortality rate was reduced from 90.6% in non-treated mice to 68.1% in the groups receiving the flavonoid therapy. In other words, only one mouse out of three survived in the group receiving bio-flavonoids. In no sense should these findings be interpreted that all citrus flavonoids exert anti-viral activity. We were unable to detect any activity in water-insoluble hesperidin or chalcone-hesperidin. On the other hand, naringin showed a slight activity against influenza virus.

During the last three years, a clinical investigation on the effect of a purified water-soluble citrus bio-flavonoid compound on virus A influenza in man was conducted. This was a small scale study covering only the cases which were proven by sero-diagnosis as true virus A influenza (Hirst test of serum-inhibition-of-hemoagglutination). 27 patients served as control. They were treated with antibiotics, if necessary, and other usual medications. No bio-flavonoids were given to them. Citrus bio-flavonoids were administered to 21 patients, 300 mg. every three hours, for four days. No other medications were given to this group of patients. Persistent fever, malaise and weakness of various degrees served as the criterion of the effectiveness of this therapy.

Although the number of cases treated with citrus bio-flavonoids was too small to make any conclusion, it appears that this therapy was beneficial in virus A influenza by reducing the feverish period of the infection from the average 4.3 days for the control group, to the average 2.2 days in the treated group.

**Discussion**

The purpose of this paper is to bring our findings concerning the antiviral activity of
citrus bio-flavanoids to the attention of Florida citrus scientists. The data we have collected, as incomplete as it might be in regard to the chemistry of active flavonoids, might stimulate their interest in this problem and might bring about new investigations along these lines. Our work is closely associated with the question of the "health angle" of citrus fruit and juice. Any claims about the "health" properties of oranges and grapefruits should be based on well-controlled and extensive experimental research. In this respect, the investigation of grapefruit flavonoids deserves the particular attention of citrus scientists. For, as we said, Dr. Cutting and associates of Stanford University, California, as well as our Virus Division, have found that naringin apparently possesses mild activity against certain viruses. If this fact is confirmed by more extensive tests, and various derivatives of naringin are investigated on their biological activity, this might be of considerable service to the Florida grapefruit industry.

REFERENCES


CHEMICAL ANALYSIS OF CITRUS BIOFLAVANOIDS

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The recent upsurge of interest in the bioflavanoids, particularly in the use of these compounds in the treatment of numerous clinical disorders, has prompted the evaluation of the available chemical techniques for analyzing the citrus bioflavanoids. So far no analytical method has shown a correlation with biological activity procedures, which in themselves are sometimes questionable methods of assay. However, there are chemical tests that are helpful in assaying bioflavanoids or extracts of same. It is the intention of this paper to describe the advantages, limitations and best adaptation of eight analytical techniques as well as to present compara-

tive results on eight citrus bioflavanoids that were analyzed by these same methods.

Experimental Procedure

Source of samples. — The following eight citrus bioflavanoids were investigated: hesperidin, naringin, hesperetin, naringenin, hesperidin methyl chalcone, hesperidin methylene carboxy chalcone, "Hesper-C" and "Citrus Vitamin P." The first four compounds were isolated in the laboratory from citrus sources and were recrystallized repeatedly to obtain highly purified products. The last four were commercial products, respectively abbreviated as HMC, HMCC, H-C, and CVP. HMC was obtained from Sunkist Growers, Inc., HMCC was another solubilized hesperidin product that represented a Florida pilot plant production, H-C and CVP were pharmaceutical products purchased locally.

Davis test. — The original method of Davis (4) was used with the following modifications. The sample was dissolved in 0.2N