Table V. Mineral Elements

element	mg/100 g of almond
potassium	766
phosphorus	364
magnesium	227
calcium	185
sodium	12.2
zinc	3.8
iron	2.6
copper	1.2
manganese	1.3
cobalt	4.8×10^{-3}
molybdenum	5.7×10^{-3}

values of analysis made on six samples.

We do not find bibliographic references concerning cobalt and molybdenum in the almond, and we suppose that these elements are hereby detected for the first time in this fruit. As is known, molybdenum is the essential element found is smallest proportions in vegetable matter. Cobalt, a component of vitamin B_{12}, is not an essential element and its presence may be attributed to associated microorganisms (Gomez Campo and Mellado, 1975).

As indicated in Table I, almonds have an ash content of 3050 mg/100 g. Potassium, phosphorus, magnesium, and calcium account for 1542 mg/100 g which is 50.56% of the ashes. The other essential elements Fe, Mn, Mo, Cu, and Zn account for 8.9 mg/100 g.

The high quantity of K, P, Mg, and Ca, together with the small proportion of Na and the content of the essential elements Fe, Mn, Cu, and Zn, allows us to consider the almond an excellent source of bioelements. According to Souty et al. (1971), calcium is only partially assimilable because it is precipited partly as an oxalate in the vacuoles of the fruit.

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Received for review August 5, 1980. Accepted December 30, 1980.

The Nature of Freeze-Induced White Spots on Orange Segment Walls

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Exposure of oranges to freezing conditions causes formation of white spots on the walls of the fruit segments. The spots are actually located in the tissue comprising the separation zone between segments; when two adjacent segments are pulled apart, each white spot is split in half. The chemical nature of the white spot material was previously in dispute, but it has now been shown to be microcrystalline hesperidin coating the walls of cells in the separation zone. Freezing causes damage to cell membranes, and a soluble form of hesperidin located in the cell vacuoles is thereby released and crystallizes.

One of the characteristic symptoms of freeze damage in oranges is the appearance of small white spots on the segment walls of the fruit. These spots have been considered for many years to be crystals of hesperidin, a citrus flavanone glycoside. Recently Albach et al. (1977) pointed out that the previous literature on the subject (Webber, 1896; Milliken et al., 1919; Hall, 1925) provided no convincing evidence that the spots were in fact crystalline or that they were composed of hesperidin. On the contrary, Albach et al. (1977) concluded on the basis of low-magnification microscopic observations that the white spots

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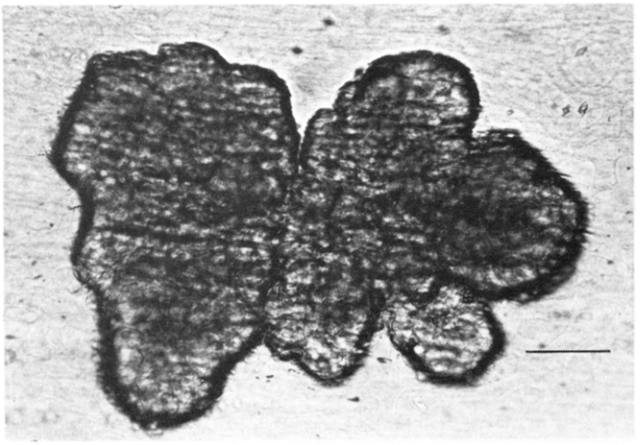

Figure 1. Photomicrograph of an orange white spot, mounted in 60% sucrose. Zeiss 10X achromatic objective; bright field; transmitted light. The bar represents 100 μm.

were probably amorphous. We have now reinvestigated this question using more suitable microscopic and microchemical techniques.

RESULTS AND DISCUSSION

During the predawn hours of Jan 2 and 3, 1979, the temperature dropped to minima of −2 and −3 °C in a grove of Valencia orange trees in Weslaco, TX. Leaf and fruit temperatures in the upper portions of the trees were undoubtedly lower than the indicated air temperature, due to radiational heat loss to the clear sky. The upper portions of these trees exposed to the open sky were defoliated and small twigs were killed. Three weeks after the freeze many of the fruit in the upper portions of these trees showed the white spots characteristic of freeze damage on their segment walls. Spots from these fruit were first examined microscopically under low magnification. By reflected light they appeared white, but transmitted light imparted a reddish brown color. With transmitted light in air, the spots were almost opaque, but in water, 60% sucrose (refractive index 1.44), and a synthetic mounting medium (refractive index 1.54) they became progressively more transparent. An object appears most transparent when its refractive index is close to that of the surrounding medium, and conversely it becomes more opaque (i.e., scatters light more strongly) as the difference in refractive index increases. Therefore, the above observations indicated that the white spot material has a relatively high refractive index. Figure 1 shows a typical white spot mounted in 60% sucrose. It is irregular in shape and does not have a crystalline appearance. The outlines of individual cells can be seen within it.

Although the spots appear to the naked eye to be on the surface of the cuticular membrane surrounding the fruit segments, they are actually embedded in the loose layers of cells comprising the separation zone (Albrigo and Carter, 1977) between segments. Figure 2 shows a cross section of a white spot between the membranes of adjacent segments. The spot is much less opaque than the one in Figure 1 because it is mounted in a medium of higher refractive index. Several layers of cells can be observed between the spot and each membrane surface. When two adjacent segments are pulled apart, each spot between them is broken in half as the separation zone is split. As a result the pattern of white spots on one segment is the mirror image of that on the other.

For closer examination under higher magnification, white spots were teased and broken up with fine needles while immersed in water. Individual cells and cell fragments could then be seen clearly (Figure 3). The white spot material appeared to be a coating on the outside surface of the cell walls. Some cells were completely covered by this material, while on others only limited areas were covered. Under highest magnification this coating was revealed to be composed of a mass of tiny particles

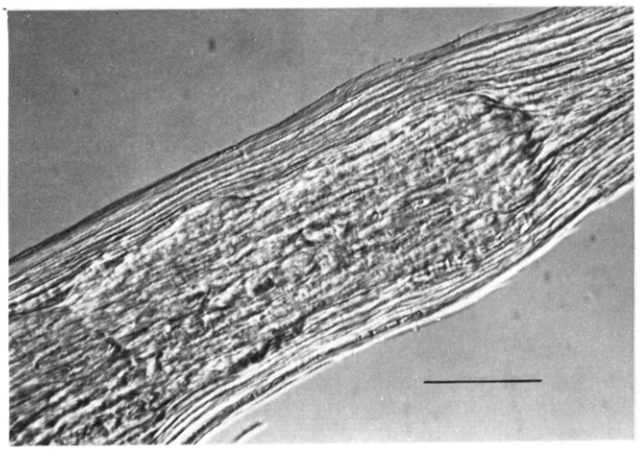

Figure 2. Photomicrograph of a cross section of an orange white spot in the separation zone between membranes of adjacent fruit segments, mounted in Histoclad (VWR Scientific, Inc.). Zeiss 16X Neofluar objective; Hoffman modulation contrast; transmitted light. The bar represents 100 μm.

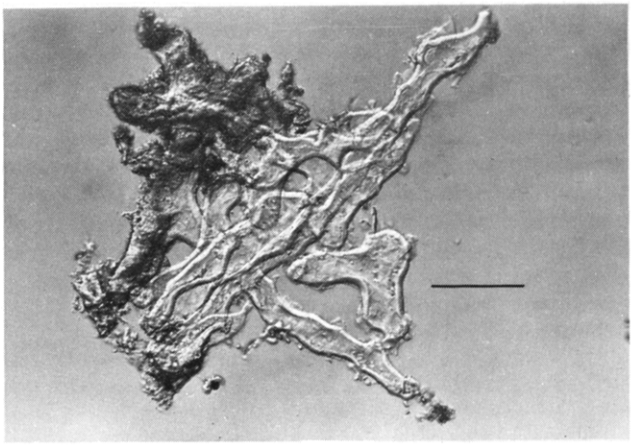

Figure 3. Photomicrograph of cells teased from an orange white spot, mounted in water. Zeiss 16X Neofluar objective; Hoffman modulation contrast; transmitted light. The bar represents 100 μm.

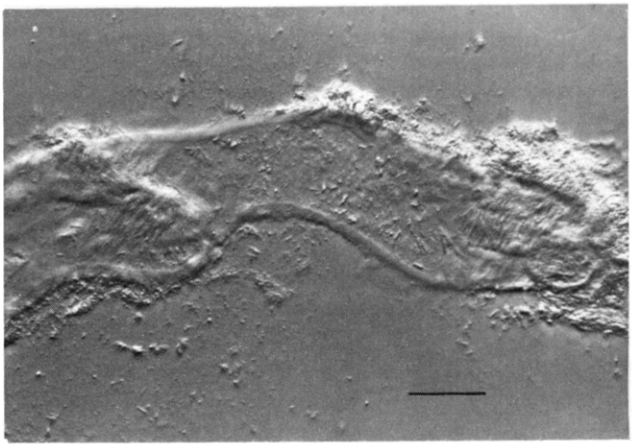

Figure 4. Photomicrograph of a cell teased from an orange white spot, mounted in 60% sucrose. Zeiss 40X Neofluar objective; Hoffman modulation contrast; transmitted light. The bar represents 20 μm.

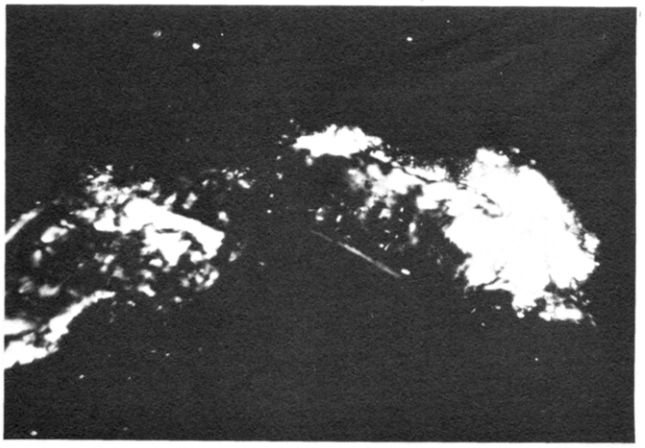

Figure 5. Photomicrograph of the cell shown in Figure 4, viewed through crossed polarizers. Zeiss 40X Neofluar objective; bright field; transmitted light.

(Figure 4). Although many of the particles were too small (less than 1 μm) to allow their shape to be determined, some of the larger ones appeared to be needle shaped, similar in appearance to hesperidin microcrystals found in citrus juices.

This observation prompted a reexamination of the evidence which led Albach et al. (1977) to conclude that the white spot material was amorphous. They observed that a white spot on a segment wall viewed between crossed polarizers appeared dark. The tissue around the spot was bright due to the birefringence of crystalline cellulose in cell walls. However, two factors make the interpretation of this phenomenon ambiguous. If the white spot material were crystalline, rotation of the plane of polarized light would be the sum of that due to cellulose in the surrounding tissue plus that due to the white spot itself. The direction and magnitude of these combined rotations could lead to extinction when the sample is viewed through the second polarizer. Furthermore, the white spot material scatters light strongly in media of low refractive index, and therefore much of the rotated light would not reach the second polarizer. In fact, we have found that in media of high refractive index white spots do show some brightness between crossed polarizers, although not as much as the surrounding tissue. In the thin cross section shown in Figure 2, the white spot had no tissue above or below it, and its brightness was almost as intense as that of the adjacent cells. When the individual cell shown in Figure 4 was examined between crossed polarizers, the white spot material was clearly birefringent (Figure 5).

Thus, the material covering the cell walls within the white spots was shown to be crystalline, but further evidence was needed to determine whether it was hesperidin. Albach et al. (1977) identified and quantitatively analyzed hesperidin by TLC in extracts of white spots cut out from segment walls. However, the white spot material is a coating on the walls of some, but not all, of the cells within the confines of a spot (Figure 3). Therefore, such an extract contains not only the white spot material but also other extractable substances from all of the cells within the area. Lacking a method for first separating the white spot material from the cells, the finding of a given constituent in an extract does not prove that it was actually derived from the white spot material. Since, therefore, the latter could not be analyzed directly, indirect evidence was sought to either confirm or deny that it was composed of hesperidin.

The solubility properties of hesperidin are unusual, in that unlike most other glycosides it is highly insoluble in water and alcohols. It is also insoluble in nonpolar solvents, as would be expected. Hesperidin dissolves readily only in very polar organic solvents, such as dimethylformamide (DMF), dimethyl sulfoxide (Me_2SO), and pyridine. Therefore, the solubility behavior of the white spots was examined, and it was found to be comparable to that of hesperidin in all respects. The spots dissolved only in DMF, Me_2SO, and pyridine, being insoluble in water and all other organic solvents tried. The solubility of the white spot material in dilute base (Hall, 1925), confirmed in this work, is also consistent with the properties of hesperidin.

The refractive index of the white spot material was next compared with that of hesperidin. The latter was immersed successively in liquids of increasing refractive index until in phenylhydrazine (refractive index 1.607) the crystals were almost invisible. Since hesperidin crystals are birefringent, they exhibit two different refractive indexes, which can be observed by proper orientation of a crystal in polarized light. By use of the Becke line test, it was shown that one refractive index was slightly less than that of phenylhydrazine and the other was slightly greater (Chamot and Mason, 1947). Since this method of determining refractive indexes requires orientation of a single crystal, it could not be applied to a whole white spot. Therefore, a white spot was immersed in phenylhydrazine and teased apart with needles, and a single crystal on a cell wall was examined. Like hesperidin, its two refractive indexes were just above and below that of phenylhydrazine.

Further supporting evidence is provided by the observation that grapefruit obtained from a grove of trees adjacent to the orange trees showed no white spots on their segment walls. Nor were white spots found in grapefruit at a grove that had been exposed to minimum temperatures as low as −5 °C during the freeze and that had been partially defoliated (upper half) by additional radiational heat loss. Even when a mature grapefruit tree was subjected to −9 °C in a portable freeze chamber (Goodier, 1979), no white spots were found in the fruit 2 weeks later. It is known that the concentrations of hesperidin are much lower in grapefruit than in oranges (Hagen et al., 1965). Grapefruit do contain large amounts of the related flavanone glycoside naringin, but this compound is much more soluble in water than hesperidin.

As a final piece of evidence, Borodin's method (Molisch, 1913) for determining the identity of microcrystalline material was employed. This method involves testing the solubility of an unknown substance in a saturated solution of a known compound. If the substance dissolves in the pure solvent but not in the saturated solution, then it is considered to be identical with the known compound. An orange white spot dissolved completely within 90 min in dimethylformamide–H_2O (9:1). When a white spot was treated with a saturated solution of hesperidin in the same solvent, however, it was still intact after 24 h. Thus, although it was not possible to isolate the white spot material for analysis, the combined weight of the evidence leaves little doubt that it is indeed hesperidin.

More than 100 years ago Pfeffer (1874) reported that hesperidin, which is highly insoluble in water, is present in a soluble form in citrus tissue. More recently, work on isolated living cells and protoplasts from citrus fruit albedo tissue has shown that this soluble hesperidin is located within the cell vacuole (Bennett and Schuster, 1978). If the cell membrane (plasmalemma) is broken, hesperidin rapidly crystallizes. (We are currently investigating the processes responsible for solubilization and crystallization of hesperidin in citrus tissue). These findings suggest that freezing of the fruit may cause cell membranes to burst

and thereby release hesperidin. An observation by C. P. Verdon at the Fruit and Vegetable Chemistry Laboratory is of interest in this respect. A navel orange purchased at a local market showed white spots on the segment walls, and the fruit had a strongly bitter taste characteristic of the triterpenoid constituent limonin. Navel oranges normally contain high concentrations of the tasteless precursor of limonin, limonoate A-ring lactone. When fruit cell membranes are broken, this compound comes in contact with an enzyme, limonin D-ring lactone hydrolase, which converts it to the bitter limonin (Maier et al., 1977). This enzymic process is slow, so normally the fruit is eaten before it becomes bitter. In the case of the fruit referred to above, its bitterness indicated that cell membranes had been broken previously. The presence of the white spots showed that the fruit had been exposed to low temperatures. These observations provide strong evidence that freezing conditions cause damage to cell membranes, which would explain the formation of white spots composed of crystalline hesperidin.

It remained to be explained why crystallization of hesperidin occurs in only certain small areas rather than uniformly throughout the tissue. This was clarified by an experiment in which a Valencia orange was immersed in ethanol for 3 days. Treatment of hesperidin-containing tissue with ethanol causes the flavonoid to crystallize within the cells in which it is present in high concentration. The segment walls from the ethanol-treated fruit showed crystallization only in local clusters of cells. The distribution of such clusters was similar to that of freeze-induced white spots. Apparently, therefore, hesperidin is unevenly distributed in the tissue, and groups of cells with high concentrations of the flavanone form white spots in response to freezing conditions.

ACKNOWLEDGMENT

We thank V. P. Maier for helpful discussions.

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Received for review October 27, 1980. Accepted February 23, 1981. References to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

Comparative Study of Whole Seed Protein and Starch Content via Cross Polarization–Magic Angle Spinning Carbon-13 Nuclear Magnetic Resonance Spectroscopy

Daniel J. O'Donnell, Joseph J. H. Ackerman, and Gary E. Maciel*

A series of nuclear magnetic resonance (NMR) spectra of whole seeds of various types was obtained by using cross polarization–magic angle spinning (CP/MAS) techniques. Select signals in the spectra provided a means of comparing the protein content relative to the starch content within a group of seed varieties. Seeds obtained from legumes were found to be high in protein content, with different legumes showing a range of protein NMR signal intensities. A series of sorghum varieties and a series of grain types were also analyzed, and the protein content of these series was compared to that of the legumes and to that of one another. The potential of ^{13}C CP/MAS NMR for the study of seeds and other intact plant materials is strongly indicated.

The need for rapid, nondestructive methods of chemical analysis of seeds has long been recognized by agronomists. The traditional method of Kjeldahl protein determination destroys the seed, as does the newer estimation using near-infrared light reflectance (Hymowitz et al., 1974). More recently, proton activation of whole and ground seeds has permitted the measurement of total nitrogen content (Dohan and Standing, 1976). However, this and other similar methods require the use of high-energy (~16 MeV) proton beams.

In the past, wide-line ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectroscopy have been used to estimate the oil content of whole seeds (Brown and Craddock, 1972; Schaefer and Stejskal, 1975; Alexander et al., 1967; Schaefer et al., 1979) in a nondestructive manner. The oil and water components of the seeds

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0021-8561/81/1429-0514$01.25/0