Studies on tomato plants were carried out in a 24-cubic-foot growth chamber maintained at a daytime temperature of 29°C, a nighttime temperature of 24°C, and a relative humidity of 50 to 70%. The light source was from 26, 8-ft VHO-Sylvania fluorescent lamps (22,000 to 24,000 luxes at plant height).

Two leaves of a four-week-old tomato plant were placed into the leaf chambers. The soil containing the roots of the plant was watered so that 70% of the soil pores were filled. The rate of apparent photosynthesis was recorded for a period of four days for both leaves. The graph shows a part of the CO_2 fixation record for two days.

During the first two days, the rate of apparent photosynthesis for the leaves increased slightly. The morning of the third day, CO_2 fixation was less after the lights came on than it was during the previous day. In less than an hour, there was a significant decrease in CO_2 fixation. Water was added to the soil and within a very few minutes CO_2 fixation by both leaves increased. The older leaf responded slower than the younger leaf. Within two hours both leaves were fixing CO_2 at a slightly higher rate than the day before. No visual symptoms of wilt occurred during the study.

The relationship of CO_2 fixation to soil suction, relative humidity, and the conductivity of unsaturated soils is being further investigated.

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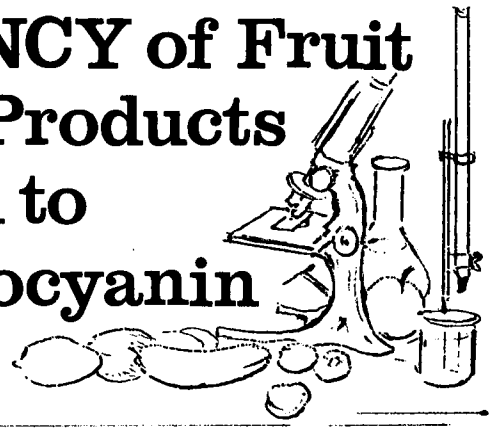
ASTRINGENCY of Fruit and Fruit Products in Relation to Leucoanthocyanin Content



M. A. JOSLYN · JUDITH L. GOLDSTEIN

THE LEUCOANTHOCYANINS occupy an important position among the water-soluble organic compounds present in the tissues of plants. They have been implicated as being responsible for the astringent taste of unripe fruits. They are responsible for the chill haze that develops in beer and for the browning of white wines. The desirable fullness of taste and body of such juices as apple, berry and grape and of fruit wines is also attributed to the astringent effect of the leucoanthocyanins. They influence the storage stability of wines and juices.

The toxicity of feeds such as Korean Lespedeza hays and carob bean meal is related to the tannins present. The presence of these compounds in fruit and plant tissue so far has been largely detected qualitatively from the anthocyanin pigment they form on heating with mineral acids. Pure leucoanthocyanin preparations have been isolated only from woody tissues, from cacao beans and recently from Japanese persimmon fruit. The chemistry and physiology of leucoanthocyanins, and related phenolics has been under investigation since 1952.

Histological examination

When examined histologically, the leucoanthocyanin and related phenolic substances were found localized largely in the vascular bundles or in isolated "tannin cells" in apple, apricot, banana, peach, pear, prune, grape, persimmon and carob tissue harvested at the immature stage, when the fruit was quite astringent. When cut, the phenolics in these tissues readily diffused out of the tannin cells so that the whole tissue became colored, when tested with specific phenolic reagents. During ripening, the total phenolic content appeared to be less, and the phenolics in the tannin cells of banana, carob, and persimmon fruit no longer diffused out.

The extractability of the fruit leucoanthocyanins in the usual organic solvents decreased in ripening. This was particularly true for methanol. The total extractable leucoanthocyanin content of unripe fruit ranged from 250 mg per gram of dry weight for green carob pods, and 200 for astringent persimmons, down to 10 to 20 for such fruit as bananas, plums, and peaches. In ripe fruit these levels dropped to 20 for carob pods, to 70 for persimmons and to 5 for bananas. While the anthocyanin pigment formed from the leucoanthocyanin of apples, grapes, peaches, and pears is cyanidin, that formed from the leucoanthocyanin of the highly astringent bananas, carob pods, dates, and persimmons is delphinidin. In green astringent cacao beans, however, the anthocyanin pigment generated is cyanidin.

Phenolic compounds

The phenolic compounds actually responsible for astringency, feed toxicity or food acceptability and stability have not been isolated and characterized. The physiological basis of the sensation of astringency also has not been defined, but preliminary observations have provided clues which are reported here because of their wide interest.

A dry puckery sensation is perceived in the mouth upon ingestion of such unripe fruits as persimmon, banana or dates. This sensation is well defined by a dry feeling in the mouth. The immediate reaction is to rinse the mouth out with water, not in thirst, but in an attempt to bring relief. Unfortunately this treatment is of no avail and the feeling of dryness or tightness persists. Although astringency is perceived in the mouth, it should be

distinguished from taste. The perception of particular tastes is restricted to definite areas of the tongue surface and epiglottis, whereas astringency is perceived over all the oral mucous membrane. Astringency differs from taste in that the threshold for perception is much greater. Previous investigators had reported that the relative astringency of substances could be determined more easily by the use of powdered preparations rather than a solution of these compounds. This is substantiated by the high concentrations of tannin (100 mg and over/100 mls) required to be detectable in wines and fruits. In contrast, leucodelphinidin from the carob bean and persimmon was astringent at 20 mg per 100 mls. Astringency again differs from taste in the time lag for perception; a longer delay than in the perception of taste and considerably longer than the familiar autonomic reflex.

Combination

Many investigators have suggested that the astringency caused by the ingestion of unripe fruit is due to the combination or binding of their phenolic compounds with the protein or mucopolysaccharide of the mucous membranes or saliva. This suggestion was made by analogy between the relative astringency of vegetable tannins and the ability of these compounds to convert animal hide into leather. The physico-chemical basis for the tanning reaction is the cross-linking of the keto-imide group of adjacent protein chains by the aromatic hydroxyl groups of the tannin, due to hydrogen bonding. For this reaction to occur, the tannin must be of the correct molecular size to cross-link adjacent collagen fibers, and there must be a sufficient number of free phenolic hydroxyl groups to achieve attachment at several points. Ionic bonds may be formed between any charged substituent, in addition to hydrogen bonds, for example between a carboxyl group and an amino group, such as occurs in lysine.

Sensation

It has not been demonstrated that the sensation of astringency perceived in the mouth is due to the combination of plant phenolic compounds with the oral mucopolysaccharides or proteins. The degree of astringency of pharmacological preparations, however, is traditionally determined by tests depending on the ability of astringents to bind or shrink protein, for example, inhibition of haemolysis of erythrocytes, permeability changes, and changes in elasticity or volume.

The dry puckery sensation perceived in the mouth is not an isolated sensation but is part of a more general syndrome. It is experienced by people suffering from *diabetes insipidus*, it occurs in conditions of severe thirst, alcoholism, or it can be induced by the action of certain drugs, for example, atropine. These observations make it likely that the sensation of astringency is part of a general physiological reaction, perhaps similar in mechanism to pain.

The tongue and oral cavity are innervated by the andrenergic nerves of the sympathetic nervous system and the cholegenic nerves of the parasympathetic system. The sympathetic nerves secrete the catecholamines, adrenaline and nor-adrenaline. These catecholamines are known to be methylated in the 3^1 position on liberation into the cytoplasm, and their biological function is thus modified. The enzyme involved is an O-methyl transferase which requires S-adenyl methionine as the methyl donor and Mg^{++}. It was found that *in vivo* pyrogallol acted as a competitive inhibitor to this system. Phenolic compounds of a leucoanthocyanin nature, containing catechol or pyrogallol nuclei, are believed to be responsible for astringency in unripe fruit. It is likely that astringency is caused by the build-up of the catecholamines in the cytoplasm because the extraneous phenolic compounds have been preferentially methylated.

Aqueous extract

It was found that the dry puckery sensation caused by an aqueous extract from persimmon, *Diospyros kaki* L. and carob bean, *Ceratonia siliqua* L. (containing 0.5 mg/ml leucodelphinidin) could be removed by rinsing the mouth out with a methionine solution (5 mg/ml) whereas water or glycine was without effect. However, if the astringent solution was mixed with methionine prior to tasting, the degree of astringency was only depressed by one unit on an arbitrary one-to-five scale.

These observations are in agreement with the hypothesis that astringency is a general physiological phenomenon similar to pain, and hence may involve an adrenergic mechanism.

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CHEMICAL *Perennial*

C. M. MC KELL · B. L. KAY

Results of this study indicate that certain herbicides may be used to reduce soil-moisture depletion by weedy plant species and thus insure good stands of grasses, even when seeded in seasons of low rainfall. Vigorous grass plants are more likely to result if soil-moisture reserves are high at the time of seeding.

ONE OF THE most crucial factors limiting the establishment of perennial grasses in areas of low precipitation is the competition for soil moisture from competing vegetation at the time of seeding. After removal of sagebrush by fire or mechanical means, weedy annual grasses offer such intense competition that attempts to establish seeded species may be unsuccessful. Most of the weedy annual grasses, including such species as cheatgrass (*Bromus tectorum* L.) and medusahead (*Taeniatherum asperum* (Simk.) Nevski), are low in desirability as forage species during some periods of the year.

From earlier work with herbicides to reduce competition during seedling establishment, three materials were chosen for these tests: ethyl *N*, *N*-di-*n*-propylthiolcarbamate (EPTC), 2-chloro-4, 6-bis (ethylamino)-*s*-triazine (simazine), and 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (atrazine). This field study was conducted in 1959–60 in Modoc County in two locations formerly occupied by sagebrush (*Artemisia* sp), but presently dominated by medusahead in one and cheatgrass in the other. Three rates of each herbicide, replicated four times, were applied as a water spray to 10×20 ft plots on September 3. A rain of 0.64 inch had occurred on August 20.

In the medusahead location, the number of replications was doubled and half the treatment areas were harrowed to incorporate the herbicides as a comparison with surface application. Gypsum electrical-resistance blocks were buried at 12 and 30 inches (in the cheatgrass site, blocks were buried at 10 and 18 inches)