Late-season Sucrose Accumulation: Can *Fact* be separated from *Fantasy*?

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Through many years there have been observations of late-season "spikes" or apparent sudden increases in sucrose percentage of sugarbeets. These observations have resulted in a widespread belief that a light frost or episode of cold weather prior to harvest causes a "sugaring up" or "ripening" of sugarbeet. For a number of physiological reasons, this is unlikely to represent a "real" or "true" accumulation of sucrose in the root. In response to the many questions I have received about this phenomenon, I offer this physiological and biochemical explanation.

Photosynthesis is the critical process underlying sucrose accumulation in any season. In late season the daylength has diminished significantly from its June maximum, temperatures are decreasing, and leaves become less photosynthetically efficient. Each of these factors individually, and all of them together, can only result in a gradual decrease in photosynthesis in the fall. Furthermore, the sugarbeet plant is making biochemical preparations for overwintering and subsequent regrowth, bolting, and flowering in the second growing season. Because for sucrose recovery purposes sugarbeet is grown as if it were an annual, harvested at the end of the first summer's growth, it is easy to forget that as winter approaches, the plant is making fall biochemical preparations necessary for survival through a winter. Part of those preparations include the storage of sucrose, but there are other biochemical processes activated in the fall that do not occur earlier in the growing season.

Physiologically, the sugarbeet root does not "ripen" in the same sense as a fruit. There is no appreciable starch or other storage product that quickly can be converted to sugar. Furthermore, a frost or light freeze can only harm leaves, not help them. As a result, photosynthesis is decreased relative to "better" conditions. Less photosynthesis means less assimilate (ultimately, sucrose) available to be stored.

The belief in "sugaring up" probably arises from one or more factors that may not necessarily be the same in each incident. A first consideration is the fact that sucrose usually is measured by polarimetry, and expressed as the percent of sugar included in a given unit of root fresh weight. Thus, a 18.0% sucrose beet means each 100 grams of root fresh weight include 18.0 grams of sucrose. That is, each 100.0 grams of fresh root sample includes 18.0 grams sucrose and 92.0 grams of "everything else"—water, cell walls, other biochemical components, etc. Expressing sucrose in this manner means that the moisture level of the root has an effect on the apparent sucrose present. Thus, rapid dehydration in late season can contribute to an "apparent" increase in percent sucrose, if it is measured by polarimetry. For example, if the 18.0% sucrose beet described above were dehydrated so that it lost just 1.0% of its fresh weight, the same 100.0 gram fresh weight sample prior to dehydration now would weigh only 99.0 grams, but it would include the same 18.0 grams of sucrose. When expressed as a percentage (grams per hundred grams), 18 g sucrose/99.0 g fr wt =

18.18 g sucrose/100 g fr wt or 18.18%. In other words, the sucrose percentage of 1% dehydrated beets increases by 1% (of the former value), and so on. The beets have not changed in sucrose, but appear to have done so because of the way the sucrose content is expressed.

An even more likely cause of an "apparent" increase in % sucrose is that in making preparations for overwintering, beets may produce several compounds that have high positive optical rotations. The polarimeter simply measures the net optical rotation of all compounds in the extract presented to it. We assume that all the rotatory compounds in the extract are sucrose, which has a high positive rotation. Under good fall conditions, this is an acceptable assumption. However, if other compounds in a sucrose extract contribute to a high positive rotation, those compounds are interpreted as if they are sucrose. Some amino acids, some other simple sugars, and some oligosaccharides such as raffinose and the kestoses are examples of compounds that would affect the polarimeter reading. If such compounds are present in significant quantity, they can lead to major errors in sucrose determination.

From a biochemical perspective, the accumulation of raffinose appears to be a very important cause of late-season sucrose analytical error. Decreasing photoperiod and temperatures during autumn stimulate frost and cold adaptation, an obvious preparation needed for the plant to survive a winter. Raffinose and other compounds related to it are associated with cold-hardiness. Importantly, galactinol synthetase, the key enzyme for raffinose synthesis, is cold-induced. In the sugarbeet literature one can find data showing that varieties differ in raffinose content at harvest, that raffinose content in a given variety differs with growing location, and that raffinose content in a variety can increase by as much as 30% when harvest periods differ by 3 weeks. My research data for raffinose content of beets at harvest and under controlled storage support those data. Biochemically, it appears that galactinol synthetase is induced when photoperiod and temperature decline, particularly with a frost. This enzyme is an essential intermediate in the synthesis of raffinose by these reactions:

(1) UDP-Galactose + *myo*-inositol —> Galactinol

(2) Galactinol + Sucrose —> Raffinose + *myo*-inositol

The first reaction is catalyzed by galactinol synthetase, discussed previously, and the second by galactinol sucrose transferase, which probably is not limiting and is not cold-induced. The important point about these two reactions is the high positive optical rotations of all the reactants except *myo*-inositol, which is optically inactive. Thus, a cold period or frost may signal the plant that it is time to accumulate raffinose as part of winter survival preparation. The cold period may induce formation of the enzyme required for the first step in raffinose synthesis, the synthesis of galactinol. Galactose, galactinol, and raffinose all have greater optical rotations than sucrose, so their combined amount is interpreted from polarimetry as even more than that amount of sucrose. Each molecule of sucrose that is lost through incorporation into a raffinose molecule appears to the polarimeter as more than one molecule of sucrose, because the optical rotation of raffinose is higher than that of sucrose by about a factor of 1.5.

It seems most likely that in addition to the normal late-season slowing of sucrose accumulation as photoperiod and temperatures decline, there often is some root dehydration (not a bad thing unless carried to an extreme so that the root is excessively stressed or wilted). When this normal physiological occurrence is coupled with the induction of galactinol synthetase, whether gradual or

as a result of a cold episode, an "apparent" late-season accumulation of sucrose occurs. It must be stressed that sucrose does continue to accumulate in late season as long as temperatures are moderate and days sunny, but the rate of accumulation gradually slows. It is a sudden increase in apparent sucrose under cold or frost conditions that requires explanation, and the explanation is a biochemical one. If we measured sucrose on the basis of dry weight and by a specific analytical technique that determines sucrose alone, not other compounds with similar optical properties, it would become clear that no "sugaring up" or spike of true sucrose accumulation occurs.

If a severe frost or lengthy freeze occurs and root structural damage results, a new set of problems occurs and vastly overwhelms the typical raffinose response to cold. Cells rupture, microorganisms proliferate, and often polysaccharide gums such as dextrans (with very high positive rotations) accumulate. In these cases, polarimetry is of almost no use in determining sucrose content and other techniques must be used.