Physiological and Biochemical Determinants of Sugar and Acid Content in Citrus Fruits and Citrus Fruit Proteomics.

Eduardo Blumwald
Plant Sciences, UC/Davis

Citrus fruit quality standards have been determined empirically, depending on species and on the particular growing regions. In general, the TSS (total soluble solids) to total acidity (TA) ratio determines whether citrus fruit can be marketed.

The TSS/TA ratio in citrus fruits is dominated by two main components: (i) overall vacuolar juice cell acidity and (ii) juice cell sugar content. The goal of this proposal is to characterize the physiological and biochemical components that promote and control the accumulation of TSS and TA in citrus fruits.

The biochemical characterization of the different fruit juice cell vacuole transporters and sugar biosynthesis pathways will open many possibilities for fruit improvement. The understanding of the physiological and biochemical determinants for TSS and TA content in fruits will allow the enhancement of fruit quality during post-harvest practices, the improvement of citrus fruit sweetness, and the characterization of physiological disorders that depend on TSS and TA fruit content. Our extensive expertise in protein biochemistry and biochemical and physiological functional analyses placed us in a unique position to aid the California Citrus Research Board efforts towards the development of Citrus Genomics Resources. Therefore, we have also taken as an aim to characterize the juice cell Proteome in order to contribute to the validation of the ESTs collections being developed under the sponsorship of CRB.

During the granting year we have made significant progress on a number of components of our proposal:

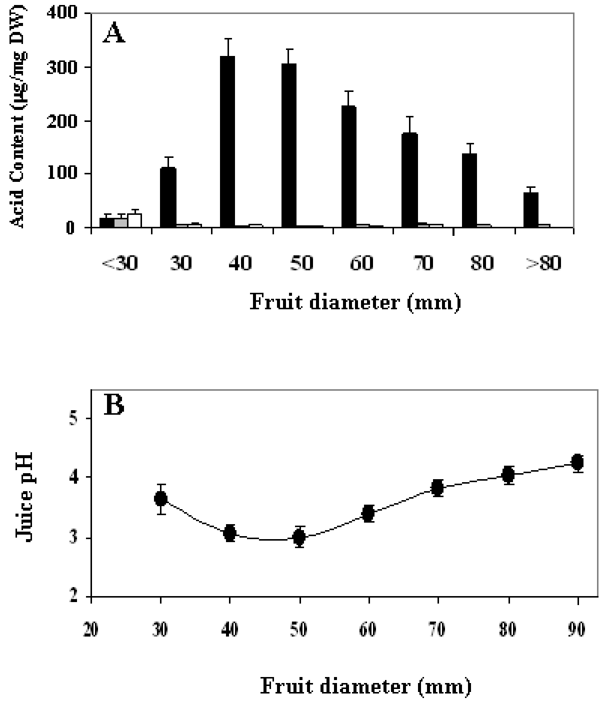

Figure 1. (A) Relative organic acid content of Washington Navel fruits were determined in juice cell sacs at different developmental stages by LC-MS using Ribitol as an internal standard. Citrate (solid bars), Malate (grey bars), Quinate (white bars). Results are the Mean ± S.E. (n = 4). (B) Juice cell pH. Values are the Mean ±S.E. (n = 5).

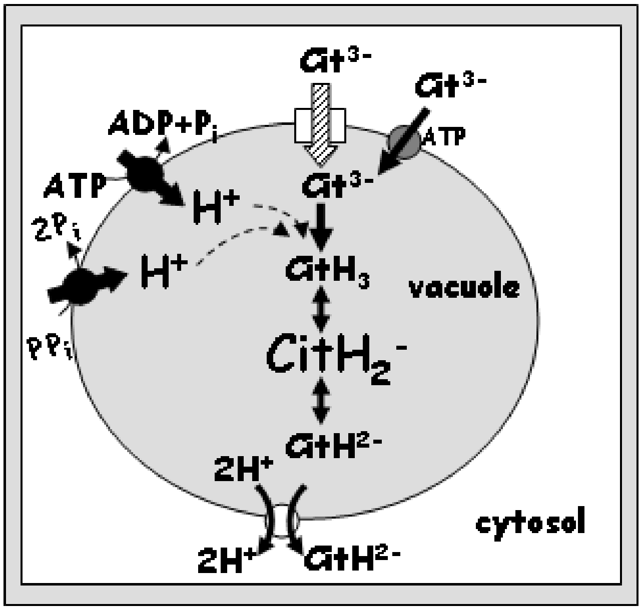

Figure 2. A simple model applicable to citrate transport in Washington navel orange juice sac cell vacuoles is shown in the figure. According to this model, citrate^{3-} enters the vacuole through inward-rectifying channels and possible through an ATP-dependent citrate transporter. Once in the vacuole, citrate^{3-} is rapidly protonated to citrateH_3 and citrateH_2^-. The alkalinization of the vacuole is avoided by the action of the H^+-PPiase and the H^+-ATPase that maintain the acidic-inside pH and citrateH_2^- as the main vacuolar buffer. During maturation of the fruit, the vacuolar pH increases with the concomitant increase of citrateH^{2-}, that is exported out of the vacuole by the action of CsCit1. The selectivity of CsCit1 for citrateH^{2-} and the low permeability of tonoplast for citrateH_2^- contribute to maintain the vacuolar buffer content and the acidic vacuolar pH.

(I) Enhancing fruit sweetness: Sucrose accumulation during citrus fruit maturation is associated with a reduction of sucrose catabolizing enzymes, sucrose synthase, and invertase. While sucrose synthase is considered to play a role in sucrose downloading from the phloem into the juice sac during most stages of fruit development, invertases are considered to play a key role in sucrose metabolism inside the juice sac cells.

We have completed the characterization of the expression of the vacuolar invertases in Washington naval oranges and identified several responsive elements in the promoter of these genes. Our basic idea is to test whether treatment of the fruits with a number of chemicals (already approved by FDA) that could activate the expression of the vacuolar invertases can alter the fructose content of the fruit, leading to an increase in fruit sweetness. If successful this approach will allow the development of simple techniques to treat the fruits either before harvesting or during packaging and storage. These treatments would result in fruits with enhanced sweetness, favoring marketing and consumer's acceptance.

(II) Manipulating fruit acidity: We have made significant progress in the development of methodology for the isolation of vacuolar membranes and intact vacuoles. These methods are the cornerstone of our efforts towards the characterization of the accumulation of acid into the juice cell vacuoles. We have identified the main citric acid transporter from Washington navel vacuoles. We have finalized our localization (in juice cells) and expression studies (using fruit slices), and completed our transport studies that demonstrate the mechanism by which the pH of the juice sac cells is regulated (Figures 1 and 2). Our next goal is to determine the factors that affect the expression (and activity) of the citrate transporter during fruit development and identify factors that could be used to change fruit acidity.

(III) The citrus fruit proteome: We have placed a considerable amount of time on the development of a citrus fruit Proteome that will serve to identify all of the proteins in the juice cells and will also serve as an aid to the Genomics efforts of the CRB (validating the annotation of the fruit genes and the different ESTs). We have already identified more than 1,000 specific fruit proteins and are now processing the information in order to build "biosynthesis maps" that will aid in defining key pathways associated with the development of key fruit quality traits.