Standardizing Postharvest Quality and Biochemical Phenotyping for Precise Population Comparison

David Rudell

Tree Fruit Research Laboratory, USDA-ARS, 1104 N. Western Avenue, Wenatchee, WA 98801

Additional index words. metabolic trait, climacteric, controlled atmosphere

Abstract. Selection of plant material with desired traits from different populations can be difficult, if not impossible, when evaluation methods are not standardized. Discerning comparable fruit postharvest traits among populations is particularly problematic because techniques and reporting protocols are often unique or non-existent for those crucial to quality and storability. Moreover, difficulties evaluating postharvest traits may be exacerbated by the dynamic nature of fruit ripening, introducing error even into intrapopulation comparisons. With the advent of biochemical phenotyping of fruit quality, opportunities to standardize evaluation of these and other important fruit postharvest traits are materializing. Standardized trait evaluation among breeding programs and, most importantly, germplasm collections is expected to allow more precise comparison between populations, expediting integration of economically important fruit quality traits into new populations as well as facilitating marker discovery.

Phenotypic comparison of plant populations within and among species is crucial for genetic research and plant breeding. Major hindrances of these comparisons include disparity among techniques used and types of data reported for each trait. Although many production-related traits are descriptive or metrics are inherently comparable, for other important product quality-related quantitative traits, including many postharvest traits, development of standardized measurement and reporting criteria can be difficult. Inconsistencies are commonly related to the different measurement and reporting protocols used, the influence of fruit maturity and/or ripeness on phenotypic comparison within and among populations, and the influence of postharvest treatment and storage regime on the final phenotype. Standardizing methodology for reporting postharvest traits would provide consistent data and information among breeding, research, and germplasm curation programs.

Postharvest traits, many of which define product storability and quality, are characterized by interactions with the postharvest environment. These include, but are not limited to, traits related to produce appearance, integrity, and overall flavor or sensory quality. Appearance is critical for many fruit and vegetable products. Traits that comprise appearance can include color, luster or waxiness, and trichome condition, all of which can be reported descriptively or quantitatively depending on the technique(s) used. Structural integrity summarizes the overall storability and appearance of fruits and vegetables and can be significantly affected by the postharvest environment. Examples include firmness and texture, water loss, chilling-related disorders, and susceptibility to pathogen infestation. Finally, flavor is a complex trait with descriptive and quantitative components. Sweetness, sourness, astringency, and aroma are common flavor descriptors, whereas measurement of sugar, acid, phenolic, or volatile component levels provide more quantitative bases for trait evaluation.

Many quantitative traits can be reduced to the individual metabolic components of which they are comprised, i.e., metabolic traits. Any single trait can be represented by a single metabolite or the interaction among many metabolites. Consequently, the complexity of the overall trait would likely dictate the influence of individual metabolites on that overall trait. For example, susceptibility of various apple cultivars to a certain type of chilling injury may vary as a result of interactions among multiple pathways responsible for injury exacerbation or amelioration (Whitaker, 2004). Conversely, changes in the levels of individual metabolites could tangibly impact the complex traits such as color, sweetness, acidity, or aroma that they comprise (Baldwin et al., 2007; Dimick and Hoskin, 1983).

Metabolic trait evaluation is a promising phenotyping technique for genetic evaluation and plant breeding strategies. Targeted and untargeted metabolic trait analysis of mapping populations is useful to discover key postharvest-related metabolic quantitative trait loci such as tomato color (Liu et al., 2003; Saliba-Colombani et al., 2001) and fruit flavor components (Dirlewanger et al., 1999; Saliba-Colombani et al., 2001; Zantor et al., 2009), including aroma (Doligez et al., 2006; Dunemann et al., 2009; Tadmor et al., 2002). Untargeted metabolic fingerprinting techniques can be used to distinguish individuals with unique quality characteristics within breeding and wild populations (Dobson et al., 2008; Schauer et al., 2005; Stewart et al., 2007). This approach has been especially productive where forward and reverse genetic techniques such as introgression have been incorporated. Thus, metabolic trait heritability is established, unraveling metabolic control by genomic comparison as opposed to the more standard approach of examining metabolic fluctuation with changes in gene expression (Baxter et al., 2005; Schauer et al., 2006).

Accurately and precisely evaluating postharvest traits presents unique technical and practical challenges. The postharvest period, which is characterized by phenotypic change, can amount to the majority of the lifespan of a fruit or vegetable. The postharvest environ-

ment is typically adjusted to maximize quality and storability by influencing key physiological events, effectively creating a different, "artificial" phenotype from one that may naturally occur. Many fruit such as pear (Sugar and Basile, 2009; Villalobos-Acuna and Mitcham, 2008) and apple (Jobling et al., 2003; Lara and Vendrell, 2003; Lelievre et al., 1995) develop optimum quality as a result of controlled ripening intentionally provoked by manipulating the postharvest environment. Control over the postharvest environment presents opportunities to explore phenotypic differences as influenced by different postharvest regimes. However, characteristic phenotypic changes during this period can make phenotypic comparison difficult, especially in populations in which little is known about selections' postharvest behavior.

The postharvest period of climacteric fruit is defined by quality-related changes stimulated by rises in ethylene synthesis that, in turn, provoke an increased respiration rate. Ripening or the degree of ripening can be influenced by maturity at the time of harvest as well as postharvest conditions. In apple fruit, harvest maturity alters both ripening timing and intensity before and after cold storage (Fellman et al., 2003; Song and Bangerth, 1996) (Fig. 1). In many fruits, the climacteric phase is also characterized by coincident significant changes in key quality-related flavor traits such as acidity and aroma (Song and Forney, 2008), whereas other traits remain unassociated or only loosely associated (Pech et al., 2008). Furthermore, changes in many quality-related traits are expressed or altered to varying degrees under certain postharvest conditions. For example, the levels and intensity of individual metabolic components of the apple fruit aroma volatile profile are significantly influenced by ripeness, harvest maturity, and storage environment (Brown et al., 1965; Patterson et al., 1974). Volatile metabolites that significantly alter aroma characteristics are linked to long-term low oxygen cold storage (Mattheis et al., 1991, 1995; Willaert et al., 1983).

Non-climacteric fruits and vegetables also undergo dramatic postharvest environment-related changes during this period. Like

Received for publication 9 Mar. 2010. Accepted for publication 9 Apr. 2010.
e-mail David.Rudell@ars.usda.gov.

Fig. 1. 'Redchief Delicious' apples harvested from 114 to 149 d after full bloom (DAFB). Respiration and ethylene evolution of three composite samples of 1 kg of fruit (or five to eight apples) were monitored twice daily to determine the green life as affected by harvest maturity. Error bars represent SE. Increasing maturity at harvest decreased the time from harvest to increases in ethylene evolution and respiration associated with climacteric ripening and increased total levels of ethylene and respiration demonstrating the variability of ripening with harvest maturity.

climacteric species, respiration and primary and secondary metabolism can change with harvest maturity or during postharvest maturation (Phan, 1987). Hypoxic conditions can provoke fermentation in many non-climacteric fruits (Joles et al., 1994; Ke et al., 1994; Petracek et al., 2002) and vegetables (KatoNoguchi and Watada, 1997; McKenzie et al., 2004; Smyth et al., 1998) generating volatile metabolites associated with unfavorable sensory characteristics, especially for fresh-cut products. Also, traits related to tissue integrity are especially important in many relatively short-lived, non-climacteric products, including susceptibility to spoilage organisms, water loss, and textural changes, all traits that can be manipulated using storage conditions (USDA-ARS, 2004).

Preharvest environment, harvest maturity, and storage environment each compound the difficulty of using single-point evaluations to compare the postharvest phenotypes of different individuals within any population. To make accurate single-point comparisons, a trait must remain relatively static over the postharvest life or maturity/ripeness properly established and the postharvest environment controlled. Optimum maturity within a popu-

lation can differ by weeks; therefore, selecting and comparing fruit at a specific maturity can be crucial to prevent obscuring or misrepresenting key postharvest phenotypic relationships among individuals within a population.

Variance resulting from different ontogeny among breeding or mapping population should be expected where ontogeny is difficult to accurately define. This is in addition to variance already present within individuals from the same selection. Fruit phenotype can be influenced by a number of preharvest environmental factors. For instance, fruit on-tree location can influence ontogeny of many temperate tree fruits, including apple (de Bruyn and van Keulen, 1968; Farhoomand et al., 1977) making it an especially important consideration when evaluating cultivars or species in which visual estimation of maturity before harvesting is difficult. All of these factors emphasize the necessity of assuring proper replication is implemented for each trait to accurately account for variance resulting from the evaluation technique as well as biological variance among individuals from the same selection.

To standardize phenotyping, techniques that produce comparable data must be used.

This is especially true in the case of postharvest analyses in which a variety of techniques can be used that generate different, incomparable categorical or continuous data for the same trait. For example, sweetness or sugar content can be measured using a variety of techniques, including taste panels evaluating actual sweetness, measuring refractive index (an estimate of total sugars), or chromatography, which quantifies each individual sweetness-related metabolic component. Likewise, external color or tissue color can be visually estimated using color cards, a variety of colorimeters, and chromatography to evaluate individual color metabolites. These examples not only produce incomparable data, but they actually measure different traits or different aspects of a more complex trait. Furthermore, techniques including taste panels, color cards, and colorimeters, although typically standardized within a particular system, lack standardization among systems. Conversely, many instruments, including gas and liquid chromatographs and refractometers, provide standardized quantitative data making biochemical phenotyping especially useful for this purpose. Techniques are optimally chosen for the type of output they provide, but considerations also include wide disparity in expense and required involvement and expertise of the analysis when choosing protocols that would be reasonable for discipline-wide use.

Although equalizing measurement and data output may seem an obvious concern, disparity among techniques is not limited to the type of instrumental or other measurement system, but also the tissue or medium measured or sample collection and preparation protocols used. Tissue or juices sampled from the different regions of the same organ can exhibit different phenotypes, including ripening pattern and intensity of associated traits (Lara and Vendrell, 2003; Rudell et al., 2000; Yamamoto et al., 1995). This can include differences in overall expression of other traits, including aroma (Buttery et al., 1988; Guadagni et al., 1971; Jia and Okamoto, 2001), phenolic compounds associated with color and astringency (Lata et al., 2009), sugars, and acidity (Taylor et al., 1993). Sample processing technique and timing may also influence specific trait evaluations. This may include anything from the degree of tissue or juice stabilization or method of sample storage used between sample collection and measurement. Differences in any of these parameters may introduce significant variation among different phenotyping programs rendering results incomparable. Standardizing sampling and preparation protocols would ensure comparable data.

Given the variety of valid, yet dissimilar, techniques available to measure any single trait, incomparable data will continue to be generated unless a concerted effort is made to standardize methodology. Efforts to standardize postharvest phenotyping should include adaptation of protocols establishing ontogeny to assure accurate and precise comparison within and among diverse populations; establish the dynamics of a specific trait during the postharvest period; and standardized sampling, storage, evaluation, and reporting protocols.

Biochemical phenotyping can provide highly standardized means to evaluate underlying metabolic components of simple as well as more complex traits. Standardization of post-harvest phenotyping will help provide comparable fruit quality databases within and across species expediting accurate evaluation of important fruit and vegetable crops.

Literature Cited

- Baldwin, E.A., A. Plotto, and K. Goodner. 2007. Shelf-life versus flavour-life for fruits and vegetables: How to evaluate this complex trait. *Stewart Postharvest Review*. 3:1–10.
- Baxter, C.J., M. Sabar, W.P. Quick, and L.J. Sweetlove. 2005. Comparison of changes in fruit gene expression in tomato introgression lines provides evidence of genome-wide transcriptional changes and reveals links to mapped QTLs and described traits. *J. Expt. Bot.* 56: 1591–1604.
- Brown, D.S., J.R. Buchanan, and J.R. Hicks. 1965. Volatiles from apple fruits as related to variety, maturity, and ripeness. *Proceedings of the American Society for Horticultural Science*. 88: 98–104.
- Buttery, R.G., R. Teranishi, L.C. Ling, R.A. Flath, and D.J. Stern. 1988. Quantitative studies on origins of fresh tomato aroma volatiles. *J. Agr. Food Chem.* 36:1247–1250.
- de Bruyn, J.W. and H.A. van Keulen. 1968. Determination of sugar and acid content in apple. *Euphytica* 17:197–201.
- Dimick, P.S. and J.C. Hoskin. 1983. Review of apple flavor—State of the art. *CRC Crit. Rev. Food Sci. Nutr.* 18:387–409.
- Dirlewanger, E., A. Moing, C. Rothan, L. Svanella, V. Pionier, A. Guye, C. Plomion, and R. Monet. 1999. Mapping QTLs controlling fruit quality in peach [*Prunus persica* (L.) Batsch]. *Theor. Appl. Genet.* 98:18–31.
- Dobson, G., T. Shepherd, S.R. Verrall, S. Conner, J.W. McNicol, G. Ramsay, L.V.T. Shepherd, H.V. Davies, and D. Stewart. 2008. Phytochemical diversity in tubers of potato cultivars and landraces using a GC-MS metabolomics approach. *J. Agr. Food Chem.* 56:10280–10291.
- Doligez, A., E. Audiot, R. Baumes, and P. This. 2006. QTLs for muscat flavor and monoterpenic odorant content in grapevine (*Vitis vinifera* L.). *Mol. Breed.* 18:109–125.
- Dunemann, F., D. Ulrich, A. Boudichevskaia, C. Grafe, and W.E. Weber. 2009. QTL mapping of aroma compounds analysed by headspace solid-phase microextraction gas chromatography in the apple progeny 'Discovery' × 'Prima'. *Mol. Breed.* 23:501–521.
- Farhoomand, M.B., M.E. Patterson, and C.L. Chu. 1977. The ripening pattern of 'Delicious' apples in relation to position on the tree. *J. Amer. Soc. Hort. Sci.* 102:771–774.
- Fellman, J.K., D.R. Rudell, D.S. Mattinson, and J.P. Mattheis. 2003. Relationship of harvest maturity to flavor regeneration after CA storage of 'Delicious' apples. *Postharvest Biol. Technol.* 27:39–51.
- Guadagni, D.G., J.L. Bomben, and J.S. Hudson. 1971. Factors influencing development of aroma in apple peels. *J. Sci. Food Agr.* 22:110.
- Jia, H.J. and G. Okamoto. 2001. Distribution of volatile compounds in peach fruit. *J. Jpn. Soc. Hort. Sci.* 70:223–225.
- Jobling, J., R. Pradhan, S.C. Morris, and N.L. Wade. 2003. Induction of chill-induced ripening in Fuji apples is a function of both temperature and time. *Aust. J. Exp. Agric.* 43:1255–1259.
- Joles, D.W., A. Collier Cameron, A. Shirazi, P.D. Petracek, and R.M. Beaudry. 1994. Modified-atmosphere packaging of Heritage red raspberry fruit—Respiratory response to reduced oxygen, enhanced carbon-dioxide, and temperature. *J. Amer. Soc. Hort. Sci.* 119:540–545.
- KatoNoguchi, H. and A.E. Watada. 1997. Effects of low-oxygen atmosphere on ethanolic fermentation in fresh-cut carrots. *J. Amer. Soc. Hort. Sci.* 122:107–111.
- Ke, D.Y., L.L. Zhou, and A.A. Kader. 1994. Mode of oxygen and carbon-dioxide action on strawberry ester biosynthesis. *J. Amer. Soc. Hort. Sci.* 119:971–975.
- Lara, I. and M. Vendrell. 2003. Cold-induced ethylene biosynthesis is differentially regulated in peel and pulp tissues of 'Granny Smith' apple fruit. *Postharvest Biol. Technol.* 29:109–119.
- Lata, B., A. Trampczynska, and J. Paczesna. 2009. Cultivar variation in apple peel and whole fruit phenolic composition. *Sci. Hort.* 121:176–181.
- Lelievre, J.M., L. Tichit, L. Fillion, C. Larrigaudiere, M. Vendrell, and J.C. Pech. 1995. Cold-induced accumulation of 1-aminocyclopropane 1-carboxylate oxidase protein in Granny-Smith apples. *Postharvest Biol. Technol.* 5:11–17.
- Liu, Y.S., A. Gur, G. Ronen, M. Causse, R. Damidaux, M. Buret, J. Hirschberg, and D. Zamir. 2003. There is more to tomato fruit colour than candidate carotenoid genes. *Plant Biotechnol. J.* 1: 195–207.
- Mattheis, J.P., D.A. Buchanan, and J.K. Fellman. 1991. Change in apple fruit volatiles after storage in atmospheres inducing anaerobic metabolism. *J. Agr. Food Chem.* 39:1602–1605.
- Mattheis, J.P., D.A. Buchanan, and J.K. Fellman. 1995. Volatile compound production by Bisbee Delicious apples after sequential atmosphere storage. *J. Agr. Food Chem.* 43:194–199.
- McKenzie, M.J., L.A. Greer, J.A. Heyes, and P.L. Hurst. 2004. Sugar metabolism and compartmentation in asparagus and broccoli during controlled atmosphere storage. *Postharvest Biol. Technol.* 32:45–56.
- Patterson, B.D., S.G.S. Hatfield, and M. Knee. 1974. Residual effects of controlled atmosphere storage on the production of volatile compounds by two varieties of apples. *J. Sci. Food Agr.* 25:843–849.
- Pech, J.C., M. Bouzayen, and A. Latche. 2008. Climacteric fruit ripening: Ethylene-dependent and independent regulation of ripening pathways in melon fruit. *Plant Sci.* 175:114–120.
- Petracek, P.D., D.W. Joles, A. Shirazi, and A.C. Cameron. 2002. Modified atmosphere packaging of sweet cherry (*Prunus avium* L., cv. 'Sams') fruit: Metabolic responses to oxygen, carbon dioxide, and temperature. *Postharvest Biol. Technol.* 24:259–270.
- Phan, C. 1987. Biochemical and physiological changes during the harvest period, p. 9–22. In: Weichmann, J. (ed.). *Postharvest physiology of vegetables*. Marcel Dekker, Inc., New York, NY.
- Rudell, D.R., D.S. Mattinson, J.K. Fellman, and J.P. Mattheis. 2000. The progression of ethylene production and respiration in the tissues of ripening 'Fuji' apple fruit. *HortScience* 35: 1300–1303.
- Saliba-Colombani, V., M. Causse, D. Langlois, J. Philouze, and M. Buret. 2001. Genetic analysis of organoleptic quality in fresh market tomato. 1. Mapping QTLs for physical and chemical traits. *Theor. Appl. Genet.* 102:259–272.
- Schauer, N., Y. Semel, U. Roessner, A. Gur, I. Balbo, F. Carrari, T. Pleban, A. Perez-Melis, C. Bruedigam, J. Kopka, L. Willmitzer, D. Zamir, and A.R. Fernie. 2006. Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. *Nat. Biotechnol.* 24:447–454.
- Schauer, N., D. Zamir, and A.R. Fernie. 2005. Metabolic profiling of leaves and fruit of wild species tomato: A survey of the *Solanum lycopersicum* complex. *J. Expt. Bot.* 56:297–307.
- Smyth, A.B., J. Song, and A.C. Cameron. 1998. Modified atmosphere packaged cut iceberg lettuce: Effect of temperature and O₂ partial pressure on respiration and quality. *J. Agr. Food Chem.* 46:4556–4562.
- Song, J. and F. Bangerth. 1996. The effect of harvest date on aroma compound production from 'Golden Delicious' apple fruit and relationship to respiration and ethylene production. *Postharvest Biol. Technol.* 8:259–269.
- Song, J. and C.F. Forney. 2008. Flavour volatile production and regulation in fruit. *Can. J. Plant Sci.* 88:537–550.
- Stewart, D., G.J. McDougall, J. Sungurtas, S. Verrall, J. Graham, and I. Martinussen. 2007. Metabolomic approach to identifying bioactive compounds in berries: Advances toward fruit nutritional enhancement. *Mol. Nutr. Food Res.* 51:645–651.
- Sugar, D. and S.R. Basile. 2009. Low-temperature induction of ripening capacity in 'Comice' and 'Bosc' pears as influenced by fruit maturity. *Postharvest Biol. Technol.* 51:278–280.
- Tadmor, Y., E. Fridman, A. Gur, O. Larkov, E. Lastochkin, U. Ravid, D. Zamir, and E. Lewinsohn. 2002. Identification of malodorous, a wild species allele affecting tomato aroma that was selected against during domestication. *J. Agr. Food Chem.* 50:2005–2009.
- Taylor, M.A., E. Rabe, G. Jacobs, and M.C. Dodd. 1993. Physiological and anatomical changes associated with ripening in the inner and outer mesocarp of cold-stored Songold plums and concomitant development of internal disorders. *J. Hort. Sci.* 68:911–918.
- USDA-ARS. 2004. *Agricultural Handbook 66*. The commercial storage of fruits, vegetables, and florist and nursery stocks.
- Villalobos-Acuna, M. and E.J. Mitcham. 2008. Ripening of European pears: The chilling dilemma. *Postharvest Biol. Technol.* 49:187–200.
- Whitaker, B.D. 2004. Oxidative stress and superficial scald of apple fruit. *HortScience* 39:933–937.
- Willaert, G.A., P.J. Dirinck, H.L. Depooter, and N.N. Schamp. 1983. Objective measurement of aroma quality of Golden Delicious apples as a function of controlled-atmosphere storage time. *J. Agr. Food Chem.* 31:809–813.
- Yamamoto, M., T. Miki, Y. Ishiki, K. Fujinami, Y. Yanagisawa, H. Nakagawa, N. Ogura, T. Hirabayashi, and T. Sato. 1995. The synthesis of ethylene in melon fruit during the early-stage of ripening. *Plant Cell Physiol.* 36:591–596.
- Zanor, M.I., J.L. Rambla, J. Chaib, A. Steppa, A. Medina, A. Granell, A.R. Fernie, and M. Causse. 2009. Metabolic characterization of loci affecting sensory attributes in tomato allows an assessment of the influence of the levels of primary metabolites and volatile organic contents. *J. Expt. Bot.* 60:2139–2154.