The three responses of plant tissue to wounding

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Abstract

We are all aware that fresh-cut produce is alive, with all its accompanying attributes, but we often fail to consider what happens to the injured tissue as it responds to being wounded. The response to wounding can be physical (dependent on the current physical make-up of the tissue), biochemical (dependent on the existing chemicals within the tissue), and/or physiological (dependent on the ability of the wounded and adjacent tissue to respond physiologically). Plant tissues have evolved an impressive, but very limited, number of physiological responses to naturally occurring injuries. These responses can be desirable (e.g., wound healing of harvested root crops, production of pharmaceutical compounds) or undesirable (e.g., lignification of vascular tissue, browning of cut surfaces). While most injuries incurred during fresh-cut preparation mimic naturally occurring injuries (e.g., cuts and abrasions), a few are so extensive (e.g., producing 'baby' carrots, cubing melons) that they can overwhelm the tissue's ability to deal with them properly and can elicit unexpected responses. The physiological response to these injuries may be mediated through a number of intermediates (e.g., wound signals, plant-growth regulators like ethylene). Understanding the limitations of natural responses and how to modulate them can be used to produce a more desirable fresh-cut product.

Keywords: antioxidants, baby carrots, fresh-cut, lettuce, off-gassing, phenylalanine-ammonia lyase (PAL), peroxidase (POD), phenolic synthesis, polyphenol oxidase (PPO), tissue browning, tissue discoloration, white blush, wound signal

INTRODUCTION

The preparation of minimally processed fruits and vegetables entails physical wounding of the tissue; e.g., apples are sliced, carrots are peeled and cut, and lettuce and cabbage are shredded. In response to wounding during fresh-cut preparation, tissue can respond as a physical object, a biochemical solution, and/or a physiologically and metabolically active tissue (Brecht, 1995; Saltveit, 1997). Often, more than one response is elicited by wounding. These unavoidable physical injuries during fresh-cut preparation cause both an immediate and a subsequent response in the tissue.

A plant cell contains many compounds that are kept in separate compartments by semipermeable membranes. A membrane surrounds the living cytoplasm of the cell and establishes a boundary between it and its external environment. The membrane surrounding the largest internal compartment, the vacuole, separates the cytoplasm, with its many enzymes, from stored organic acids and phenolic compounds. Wounding not only physically damages these membranes, but also disrupts their function so that incompatible compounds mix, producing unwanted and uncontrolled reactions. For example, phenolic compounds from the vacuole mix with enzymes in the cytoplasm to produce a brown compound that discolors the tissue.

During respiration, plant cells use O_2 from the atmosphere and food within the cell to produce energy, CO_2, and small molecular fragments that serve as substrates for future synthesis. In most tissues, if the O_2 concentration within the tissue falls below 2%, or if the CO_2 concentration rises above 5%, the predominant respiratory reactions within the tissue may change from aerobic to anaerobic, and the tissue will undergo fermentation and produce compounds that may give the product an undesirable flavor and aroma (Brecht,

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1995).

Oxygen diffuses into the tissue, and CO_2 and heat diffuse out. The rate of O_2 and CO_2 diffusion is governed by the internal concentration gradient and by the resistance of the surface (cuticle and epidermis) and internal tissues to gas movement. The resistance of the surface often has a pronounced effect on the rate of diffusion. The gradient of CO_2 across this resistance must be great enough to drive the diffusion of all the CO_2 produced by respiration. If the gradient is insufficient to produce this flux, the internal CO_2 concentration will increase until a steep enough gradient is established. Temperature affects not only the rate of respiration, but also the rate of gas diffusion. However, the effect on respiration is much more pronounced, so that, as the temperature increases, the concentration of O_2 decreases and the concentration of CO_2 increases within tissue.

TISSUES RESPOND TO WOUNDING AS A PHYSICAL OBJECT

The immediate physical effect of wounding is to cause mechanical shocks to the tissue, to remove the protective epidermal layer, to deposit surface moisture, and to expose tissue to contaminants (Table 1) (Saltveit, 1997). Later, as the surface water evaporates and the tissue starts to respond physiologically, there is a further alteration in gas diffusion and surface appearance (Table 2). Some of the immediate and subsequent physical effects of wounding are listed below.

Table 1. Immediate physical effects of wounding.

Mechanical shock to tissue	Removal of protective epidermal layer	Liquid on cut surface blocks pores	Exposure to contaminants
Bruises, cracks, fractures Hydraulic shock propagated through tissue	Alter gas diffusion Provide entry for contaminants	Reduced gas diffusion Elevated CO_2 Reduced O_2 Accelerates water loss Provides substrate for microbes	Microbial Chemical

Table 2. Subsequent physical effects of wounding.

Elimination of natural barriers	Surface debris changes appearance
Enhanced gas diffusion Reduced CO_2 Elevated O_2 Accelerated water loss Contamination	White blush formation

Wounding severely affects the resistance of the surface by removing the natural barriers and wetting the surface. Since gas diffuse through water is only $1/10{,}000^{th}$ as fast as through air, even a thin layer of water on the surface will dramatically effect gas diffusion across the surface. After wounding, surface resistance to gas diffusion initially increases and later decreases, as surface water is first deposited and then lost, and as the tissue's healing processes are activated. The concentration of O_2 and CO_2 within the tissue is also affected by the rate of O_2 uptake and CO_2 production by the internal tissue.

The development of 'white blush' on 'baby carrots' is a good example of the response of the tissue as a physical object (Cisneros-Zevallos et al., 1997). Long carrots are cut into short segments (ca. 5 cm long) and the segments are abraded or 'polished' to round the edges by removing the external few millimeters of the surface. The remnants of cell walls left over from this abrasion of the segments are appressed to the surface by the surface tension of water, and this debris is rendered transparent by water on the surface. Evaporation of this

surface water allows these wall fragments to form a dense mat that gives the surface a whitish appearance. Re-wetting the whitish segments reverses this effect. No wound-induced biochemical or physiological changes are involved.

Another example of wounded tissue acting as a physical object is the off-gassing of internal CO_2 following cutting of bulky fruits and vegetables. The cuticle and epidermis of fruits and vegetables impose a resistance to the diffusion of gases (e.g., CO_2). The internal concentration of CO_2 must be much greater than ambient to provide the necessary gradient to drive the diffusive exchange of all the respired CO_2 across the barrier. Much of the internal CO_2 is in equilibrium and dissolved in the liquid content of the tissue. The solubility of CO_2 in the cellular aqueous solution is very high (e.g., about 1mL CO_2 can dissolve in 1mL water at 15°C). Removal of the barrier to diffusion by segmenting a bulky commodity (e.g., an apple or tomato fruit) results in a massive efflux of CO_2, as the gas previously dissolved in the cells vaporizes (the physical response). Only later does a rise in CO_2 efflux represent an actual wound stimulation of respiration (the physiological response) (Brecht, 1995; Saltveit, 1997).

TISSUES RESPOND TO WOUNDING AS A BIOCHEMICAL SOLUTION

Cells are complex aqueous solutions of many reactive biochemical compounds that are rendered relatively stable by intervening systems of cellular membranes. Mechanical damage can increase membrane permeability in the injured and adjacent cells. Phenolic compounds in the vacuole can react with amino acids and sugars in non-enzyme Maillard and enzyme- (e.g., polyphenoloxidase) assisted oxidations to form ortho-quinones, which polymerize to form brown pigments. Increasing membrane stability, excluding O_2, and inhibiting enzyme activity reduce this source of browning. However, since these browning reactions occur because of the mixing of pre-existing substrates in the vacuole with enzymes in the cytoplasm, they are very rapid, and require almost immediate intervention after wounding to curtail the reactions that result in tissue browning (Saltveit, 1997; Jacobo-Velázquez et al., 2011).

The cut surface of some fruits and vegetables may brown rapidly due to the oxidation of pre-existing phenols to ortho-quinones, which in turn quickly polymerize to form brown pigments. Artichokes and apples are good examples of products that are naturally high in phenolic compounds, and will brown rapidly when injured tissue (e.g., at the edge of a cut) is exposed to O_2 in the air (Nicolas et al., 1994). This browning is an oxidation of existing phenolic compounds that is facilitated by enzymes [e.g., peroxidase (POD), polyphenol oxidase (PPO)] already in the cells. No wound-induced physiological response is necessary for this browning to occur, since it is the result of the oxidation of pre-existing compounds (e.g., phenolics). This type of browning can be mitigated by selecting cultivars with low levels of endogenous phenolics, by cultural practices that reduce the accumulation of phenolic compounds before harvest, by the immediate application of antioxidants, by inhibiting enzymes of phenolic oxidation (e.g., POD, PPO), and by the exclusion of O_2 from around the wounded tissue (McEvily et al., 1992; Nicolas et al., 1994).

TISSUES RESPOND TO WOUNDING AS PHYSIOLOGICALLY ACTIVE TISSUE

In contrast to these first two reactions of tissues to wounding (i.e., as physical objects, or as biochemical solutions), the third response involves the active participation of the tissue. The physiological and metabolic responses to wounding require the production, transmittance, and perception of unique 'wound' signals (Cisneros-Zevallos et al., 2014). Within seconds of wounding, a signal is produced in wounded tissue that propagates into adjacent tissue and induces myriad responses. These induced responses are often detrimental to the quality of fresh-cut produce. Some of the immediate physiological effects of wounding are listed below (Table 3).

Table 3. Immediate physiological responses to wounding.

Wound signal (nature, speed)	Membrane depolarization	Membrane disorganized	Loss of protoplasmic streaming
'Wound' hormone (e.g., traumatin) Wall fragment Hydraulic wave Bioelectrical wave	Increased permeability Mixing of cellular compounds Calcium and signal transduction Vacuole contents mix with cellular	Lipids oxidized Free fatty acids produced	Efflux of calcium Depolymerization of cytoskeleton

The cutting and abrasion of tissue produces a wound signal that is thought to be responsible for the induction of many wound-induced physiological responses. The nature of the wound signal and the method of its propagation from the site of injury are unresolved. Many candidates for the wound signal have been proposed: e.g., reactive oxygen species (ROS), ethylene (C_2H_4), hydraulic shock, waves of electrical potential, salicylic acid, jasmonic acid, cell-wall fragments, products of membrane oxidation (León et al., 2001). However, the transitory nature of the signal, its apparent lability, and the possibility that different species may use different methods of signaling have all conspired to make this a challenging problem. Though difficult to solve, it is becoming increasingly obvious that a better understanding of the wound signal is necessary in order to control its impact on the quality of fresh-cut products (Jacobo-Velázquez et al., 2015).

The wound signal appears to propagate from the site of injury into adjacent tissue. This progressive movement can be measured as the appearance of wound-induced enzymes or products [e.g., phenylalanine-ammonia lyase (PAL) activity and phenolic compounds] in tissue near the site of injury. Wounding lettuce tissue stimulates the de novo synthesis and activation of phenolic metabolism. The activity of PAL is a rate-limiting step in phenolic metabolism. The activity of PAL in tissue next to a cut increases within 4 h, while it takes 6 and 8 h to increase in tissue 1 and 2 cm away, respectively. This suggests that the wound signal is moving at a speed of about 0.5 cm h^{-1} in excised lettuce leaf tissue.

Most lettuces have low levels of phenolic compounds at harvest (they brown very slowly after wounding). Wounding and ethylene both promote PAL activity and the accumulation of phenolic compounds and tissue browning. If ethylene was the cause of tissue browning, simply eliminating its wound-induced increase in synthesis or action would eliminate many unwanted wound responses in fresh-cut produce. However, it appears that ethylene is not the wound signal for the induction of phenolic metabolism. A kinetic study of induced phenolic metabolism by both wounding and ethylene showed that the wound response does not involve ethylene, but is initiated by a de novo-synthesized wound signal that diffuses into adjacent tissue and stimulates phenolic metabolism. The induction of PAL activity in lettuce tissue was more rapid in wounded tissue than in ethylene-treated tissue. If wounding acted through the induction of ethylene, the level of PAL in lettuce tissue exposed to ethylene should have been higher than in wounded tissue, since the step in which wounding induced ethylene production was bypassed. In the case of lettuce, elimination of wound-induced ethylene production would therefore have no effect on the induction of PAL (Tomás-Barberán et al., 1997).

The response of tissue to wounding usually increases as the severity of the injury increases; as more tissue is injured, there is more of a response. For example, increasing the number of pin pricks in lettuce increases the response of the tissue; e.g., the level of PAL activity. However, after a certain level of injury has been reached, additional injuries cause less and less of an additional response. This probably happens because the expanding areas of induced tissue start to overlap. Since the response of individual cells appears to be limited, induction from two signals may be similar to that from one.

These overlapping areas of induced tissue can be thought of as expanding circles. Cells near the site of injury experience the greatest induction for the longest period of time. As the wound signal moves outward, its strength appears to dissipate, so cells some distance from the site of injury are induced less and for a shorter period of time. When wound signals converge from two directions, there is a double induction, but, because of the distances and limitations of the tissues to respond, there is only a slightly increased physiological response. Following the propagation of the wound signal through the tissue, there is a subsequent response that entails many physiological, biochemical and morphological changes.

The increases in respiration and ethylene production are rapid and important responses to wounding. These increases may occur through the uncontrolled mixing of cellular components (e.g., disruption of the semipermeability of membranes) or through controlled cellular repair mechanisms. Both processes produce heat, which may increase the tissue temperature. Wounding may thereby increase both the basal rate of heat production and the amount of heat produced because the tissue is metabolizing faster at an elevated temperature. Although often transitory in nature, this possible increase in heat production should be taken into account when designing packaging and storage conditions for fresh-cut produce to prevent elevated temperatures, which will shorten shelf-life.

Browning is a severe problem, and the browning potential of many tissues is affected by their prior treatment. Stresses (e.g., temperature, physical injury, disease) tend to increase the production of many phenolic compounds that brown easily upon injury. Being able to predict the browning potential before processing would help in marketing decisions and in deciding which treatments, packaging, and storage conditions would be needed to maintain maximal quality and shelf-life (McEvily et al., 1992). However, in fresh-cut produce that browns slowly, the initial PAL activity and phenolic content are poor predictors of future browning potential. In these tissues, it is the induction of enzymes of phenolic synthesis, the accumulation of phenolic compounds, and their subsequent oxidation that determine whether browning will be minimal or severe. We currently know too little about the interactions of these processes in many tissues to use their initial states to accurately predict their future.

CO_2 production does increase in tissue undergoing wound repair, as respiration is stimulated to furnish not only energy, but also molecules to synthesize the needed repair compounds. The substrates used in these reactions are often the very compounds that are prized components of quality, e.g., sugars and organic acids. The reduction and interconversion of these compounds during metabolism can significantly reduce quality. For example, the preferential respiration of organic acids can alter the sugar-to-acid ratio, making the produce insipid. Other respiratory reactions accelerate softening of some tissues and the toughening of others. The breakdown of cell-wall components produces soft tissues, while the synthesis of lignin strengthens the cell wall of fibers, making the tissue tough and stringy.

Wounding also activates the ethylene biosynthetic pathway in many commodities. Large quantities of ethylene can be produced by some injured tissues. However, not all tissues respond to wounding with increased ethylene production. Many vegetative or immature tissues (e.g., broccoli, cabbage, celery, and lettuce) normally produce small amounts of ethylene, and wounding causes only a small and transitory increase in production. Although exposure to ethylene adversely affects most fresh-cut commodities, wound-induced ethylene production will probably not be a major problem unless the wounded tissue is confined in a small, unventilated volume.

There are other crops, however, in which wound-induced ethylene production could induce significant changes. Many fruits are climacteric (e.g., apple, avocado, banana, melon, and tomato) and experience increased respiration and ethylene production during ripening. Exposure of mature, pre-climacteric fruit tissue to ethylene stimulates ethylene production and ripening. The self-stimulation of ethylene production by ethylene means that a short exposure to ethylene as the result of injection of ethylene into the storage atmosphere – as is done to ripen bananas and tomatoes – or as the result of wounding could trigger additional ethylene production and possible unwanted physiological activity, e.g., ripening or softening.

TISSUES RESPOND TO WOUNDING IN COMPLEX WAYS

This short article has only touched on a few of the many responses and interactions that occur during and after processing of fresh-cut products. For example, unexpected responses may occur. Experiments to develop a method to deliver slices of ripe tomatoes from California to distant markets encountered an unexpected problem. Mature-green tomato fruit were sliced and arranged in packages. It was planned to induce normal ripening in transit. However, slicing the fruit altered the water activity and internal gas composition around the slices and stimulated a response that had been eliminated during domestication and breeding of commercial cultivars. As the slices ripened, the seeds exposed on their surfaces began to germinate. This process of vivipary (i.e., the germination of seeds while still in the fruit) produced ripened slices covered with the roots of germinated seeds, which had the appearance of myriad white worms, an altogether unappealing apparition.

CONCLUSIONS

Wounding associated with the preparation of fresh-cut fruits and vegetables induces many physical and physiological responses. Many of these changes are unwanted and decrease product quality. The best way to control these changes is to maintain low temperatures during storage and marketing. Selecting quality starting material is another way to ensure a quality product. Post-processing treatments may be necessary to control problems such as browning, white blush, disease and textural changes. When possible, these types of treatment should be avoided, since they add cost and complexity to an already complex system.

Not all tissues respond similarly to wound-induced browning. Tissues that brown rapidly have high levels of endogenous phenolic compounds, low levels of antioxidants, adequate PPO and POD activity, and 'leaky' membranes that allow the mixing of substrates and enzymes. In contrast, tissues that brown slowly have low levels of preformed phenolic compounds, high levels of antioxidants, low levels of PPO and POD activity, and intact membranes. Different procedures should be used to prevent browning in the two tissue types. In tissues that brown rapidly, it is effective to reduce levels of phenolic compounds, (e.g., genetic selection, cultural practices), add antioxidants, exclude O_2, reduce PPO and POD activity, and increase membrane stability (e.g., with applications of calcium salts). For tissues that brown slowly, effective control measures include preventing phenolic accumulation by reducing the wound signal, by interfering with the synthesis or bactivation of enzymes and phenolic synthesis, by increasing membrane stability, and later by excluding O_2 in the package. Knowing whether a specific fresh-cut problem is the result of physical, biochemical, and/or physiological changes gives us the opportunity to target specific treatments to enhance the beneficial effects and diminish the deleterious effects of wounding.

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