The Use of Controlled Postharvest Abiotic Stresses as a Tool for Enhancing the Nutraceutical Content and Adding-Value of Fresh Fruits and Vegetables

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ABSTRACT: This paper proposes a concept based on applying postharvest abiotic stresses to enhance the nutraceutical content of fresh fruits and vegetables. We hypothesize that selected abiotic stress treatments, such as wounding, phytohormones, temperature, ultraviolet light, altered gas composition, heat shock, and water stress, among others, will affect the secondary metabolism of fresh produce and increase the synthesis of phytochemicals with nutraceutical activity or reduce the synthesis of undesirable compounds. Controlled stresses may be used as tools by the fresh produce industry to enhance the health benefit properties of fresh-cut or whole fresh produce and by the food processing and dietary supplement industries to obtain healthier processed products or enhance extractable nutraceutical yields.

Keywords: abiotic stresses, fruits, vegetables, nutraceuticals, value-added, postharvest

Introduction

The trend among consumers is to associate high quality products with a healthy diet, safety, and convenience. The fresh fruit and vegetable industry is an important contributor to the U.S. economy, with estimated sales in 1999 of approximately $76 billion. The fresh-cut industry sales represented $10 to $12 billion in 2000, with a growth projection of 10% to 15% per year for the next 5 y in the U.S. retail market (Garret 2002). Additionally, there is an increasingly growing market for nutraceuticals and functional foods. Products containing nutraceuticals have reached a worldwide estimated value of $65 billion (Lachance 2002). In the U.S. alone, this figure is approximately $24 billion with an expected rise to $35.4 billion by 2006 (Datamonitor 2002). Within the nutraceutical category are antioxidants, such as vitamins C and E, carotenoids, and phenolic compounds, among other plant secondary metabolites with well-known and documented preventive roles against certain cancers and cardiovascular diseases (Scheerens 2001). These antioxidants can be used for controlling degenerative oxidation reactions caused by reactive oxygen and free radical species in living tissues and the inhibition of lipid peroxidation in foods caused by processing and during storage (Halliwell and others 1992, Yen and others 1997). A recent review on nutraceuticals and human health has been presented by Dillard and German (2000).

Enhancing the health benefit properties of fresh produce will add value and create new opportunities for growers and processors by reaching these health-oriented markets. To achieve this goal, there is a need to provide technologies that can ensure the delivery of high quality products with high levels of the desired nutraceuticals.

Plants, when exposed to unfavorable environments, such as water deficit, chilling, heat stress, oxygen deficiency, and air pollution, result in some degree of stress and express only a fraction of the plants’ genetic potential. Plants adapt to unfavorable conditions through genetically determined stress resistance (Drew 1998). Abiotic stresses will affect the pathways involved in the biosynthesis of the 3 principal groups of secondary metabolites: terpenes, phenolics, and nitrogen-containing compounds. These compounds serve plants as defenses against herbivores and pathogens, as attractants for pollinators and seed-dispersing animals, and assist with absorbing harmful ultraviolet radiation or reducing the growth of competing plants among others (Gershenzon 1998). Due to their biological activity, secondary metabolites are used commercially as insecticides, fungicides, pharmaceuticals, fragrances, flavorings, medicinal drugs, and industrial materials (Gershenzon 1998). This has stimulated the need to design strategies for their enhancement in crop tissues, such as the proposed classical breeding (Connor and others 2002a, 2002b), optimization of production practices (Kalt and others 2001), or genetic engineering (Kochian and Garvin 1999, Winkel-Shirley 2002).

Abiotic stresses can be used in preharvest activities to enhance the quality and yield of products in the field (Kalt and others 2001). For example, vitamin C can be enhanced in plants exposed to higher light intensity or those with less frequent irrigation (Lee and Kader 2000). Pungency levels can be increased in water-stressed pepper fruits (Estrada and others 1999), whereas apple skin color may be improved by applying ethephon and phosphorus-calcium mixed compounds 4 wk before commercial harvest (Li and others 2002). Abiotic stresses are used in postharvest activities as traditional tools to extend the shelf life of products, focusing mainly on color, texture, and flavor quality changes (Kader 1992). Among the stresses used are wounding for fresh-cut (Saltveit 1997), altered O2 and CO2 levels in controlled and modified atmospheres, or C2H4 gassing for ripening and degreening (Kays 1991).

Postharvest abiotic stresses may affect the levels of secondary metabolites in crop tissues. For example, anthocyanin accumulation due to ripening has been reported for thornless blackberries (Sapers and others 1986), apples (Curry 1997, Faragher and Brohier 1984, Arakawa 1991), and strawberries (Given and others 1988).
Temperature will affect anthocyanin content in strawberries (Missczak and others 1995) and in colored potatoes during cold storage (Lewis and others 1999). Wounding will increase phenolic acids and anthocyanins in red pigmented lettuce (Ferrerres and others 1997) and phenolic compounds in carrots (Babic and others 1993a, 1993b; Howard and Griffin 1993). Phytohormones, such as ethylene will induce phenolic compounds in carrots (Lafuente and others 1996) and lettuce (Ke and Saltveit 1988). Methyl jasmonate will induce anthocyanin accumulation in tulip bulbs (Saniewski and others 1998) and promote β-carotene accumulation in Golden Delicious apples (Perez and others 1993). Light induces the accumulation of chlorogenic acid in potato tubers (Percival and Baird 2000), anthocyanin in red cabbage (Craker and Wetherbee 1972) and apples (Tan 1979; Dong and others 1995), and querecitin in Granny Smith apples (Reay 1999).

These abiotic stresses affect phytochemical accumulation or loss by inducing an increase or reduction in key enzyme activities of secondary metabolic pathways (Dixon and Paiva 1995). For example, in the phenylpropanoid pathway, phenylalanine ammonia-lyase (PAL) is induced in lettuce by ethylene, generating an increase in phenolic compounds (Hyodo and others 1978). However, not all the abiotic stresses are beneficial. Some stress treatments may induce phytochemical loss or induce production of undesirable compounds. Carbon dioxide has been shown to promote anthocyanin loss in stored pomegranate arils (Holcroft and others 1998) and strawberries (Gil and others 1997). Temperature may induce chlorophyll loss in broccoli (Toivonen and Sweeney 1998). Heat treatments may reduce PAL activity and anthocyanin accumulation in strawberries during storage (Civello and others 1997) and phenolic synthesis in lettuce (Loaiza-Velarde and Saltveit 2001). Water stress may induce ascorbic acid loss in strawberries (Nunes and others 1998) and leafy greens (Lazan and others 1987). Ethylene may induce the bitter compound, isocoumarin, in carrots (Lafuente and others 1996) and the bitter compound, xanthotoxin, in parsnips (Shattuck and others 1988). Light and wounding may stimulate glycoalcaloid synthesis in potato tubers (Salunkhe and Wu 1979), whereas γ irradiation may reduce chlorogenic acid synthesis in wounded potatoes (Pendharkar and Nair 1987).

All these studies indicate that there is a potential in using stresses to induce accumulation of targeted phytochemicals, thereby enhancing the genetic potential of fruits and vegetables and yielding products with increased health benefit properties. However, there are only few references in the literature reporting the use of controlled stresses to enhance the accumulation of nutraceuticals. For example, Kalt and others (1999) showed that storing different types of berries at temperatures > 0 °C induced phenolic synthesis and increased the fruit total antioxidant capacity. Cantos and others (2000, 2001, 2003) found that using UV irradiation enriched the resveratrol content in red table and wine grapes. Recently, our group has shown that different postharvest abiotic stresses such as methyl jasmonate will increase the anthocyanin content in purple-fleshed potatoes (Reyes and others 2001) and differentially increase phenolic compounds, avoiding the accumulation of isocoumarin phytoalexins in purple carrots (Heredia and others 2001). Moreover, we have shown that wounding increases the phenolic content and the antioxidant capacity in orange carrot tissue (Heredia and Cisneros-Zevallos 2002). Similar response to wounding for increased antioxidant capacity was reported later for lettuce tissue (Kang and Saltveit 2002).

In this paper, we hypothesize that applying controlled postharvest abiotic stresses to fruits and vegetables can enhance their nutraceutical levels. This concept can be used as the basis to develop a novel tool that the fresh produce industry can use to enhance the health benefit properties of fresh produce. This approach also may be used by the functional food and dietary supplement industries to obtain healthier processed products or for the enhancement of extractable nutraceutical yields.

**Experimental Approach**

To support this hypothesis, we will use experimental data presented at the 2001 and 2002 Annual Meetings of the Inst. of Food Technologists (Reyes and others 2001; Heredia and others 2001; Heredia and Cisneros-Zevallos 2002). The 1st set of data shows the effects of phytohormones on the phenyl-propanoid metabolism in whole potatoes and sliced carrot tissue, evaluating the accumulation of anthocyanin and other phenolic compounds. Purple-fleshed potatoes “All Blue” grown in Springfield and Rio Grande City (Texas) and purple carrots “Betasweet” purchased in Texas were treated with methyl jasmonate (4.59 mmol) or ethylene (1000 ppm). Potatoes were stored for 10 to 14 d and carrots for 5 d at room temperature in dark using closed 4-L jars that were periodically ventilated (Reyes and others 2001; Heredia and others 2001).

The 2nd set of data shows the effect of wounding on the antioxidant capacity in purple-fleshed potato and orange carrot tissue. Disks, 3-mm thick, were sliced from both tissues with a sharp blade and stored at room temperature in dark for 2 d using closed 4-L jars that were periodically ventilated (Reyes and others 2001, Heredia and Cisneros-Zevallos 2002).

In all experiments we ensured that CO₂ was < 0.5% and that weight loss was not > 2%. Total anthocyanins were determined using a spectrophotometric method according to Fuleki and Francis (1968) and expressed as mg cyanidin-3-glucoside/100 g tissue (ε = 25965 cm⁻¹M⁻¹; Abdel-Aal and Hucl 1999), whereas total phenolic content was calculated by the Folin–Ciocalteu phenol reagent assay (Hyodo and others 1978; Talcott and Howard 1999) and expressed as mg chlorogenic acid/100 g tissue. The antioxidant capacity of extracts was performed by reaction with a stable radical, 2,2-Diphenyl-1-picrylhydrazyl, in a methanol solution (Brand-Williams and others 1995). Results were compared with a standard curve prepared with Trolox (6-Hydroxy-2,5,7,8-tetramethylchro- man-2-carboxylic acid) and expressed as μgTrolox/1 g tissue.

**Results and Discussion**

**Interaction among abiotic stresses**

Understanding how crop tissues respond to different abiotic stresses, applied alone or in combination with others, would be the basis for designing strategies to enhance nutraceutical levels in tissues.

It is known that plant tissues have the capacity to acclimate, by improving stress resistance (Drew 1998, Brodli 1990). The heat shock stress in tissues is an example in which tissues respond by synthesizing heat shock proteins. The precise role of these proteins remains unknown, but they appear to act as protective agents against further stresses (Vierling 1991). Some heat shock proteins are not unique to high temperature stress and can be induced by a wide variety of environmental stresses or conditions (Bonham-Smith and others 1988). That stressed tissues gain cross-protection against other stresses was shown in an experiment with lettuce, where a heat shock treatment was a higher priority than wounding in terms of affecting metabolism (Saltveit 1997).

Understanding the interaction among stresses will make it possible to find practical applications. For example, the hierarchical response structure for stresses could be used to modulate the tissue response to other stresses by preventing it or attenuating it (Saltveit 1997). Alternatively to a hierarchical response, we might
see an additive response or a synergistic response, which might lead to increasing nutraceutical levels in the crop tissue. Synergistic effects in anthocyanin induction have been reported with redlight and low temperature stresses in sorghum seedlings (Shichijo and others 1993), with jasmonic acid and light irradiation applied to Vitis vinifera suspension cultures (Zhang and others 2002), and with ethephon and sucrose treatments in grape berries (Roubelakis-Angelakis and Kliever 1986). Furthermore, the sequence of applied stresses may have an impact on phytochemical accumulation, as in the case of temperature and red-light exposure influencing the accumulation of anthocyanins in red cabbage seedlings (Small and Pecket 1982). However, more studies are needed to understand the different interactions.

We found that wounding alone in purple carrot tissue increased phenolic content by about 117% compared with day 0. When wounding was applied in combination with ethylene or methyl jasmonate, the total phenolic content increased by about 176% and 210%, respectively (Figure 1). The response of carrot tissue to the combination of wounding and phytohormones suggests an additive response and opens the possibility of practical use for increased nutraceutical levels in sliced carrot tissues. However if undesirable compounds are being synthesized, it will affect the selection of the appropriate stress or combination of stresses. For example, the combination of wounding and ethylene led to a higher level of the bitter compound, isocoumarin, in the tissue (approximately a 10-fold increase) compared with day 0. Wounding stress alone or combined with methyl jasmonate only increased isocoumarin levels by approximately 0- and 2.3-fold, respectively, which is below the level of sensory detection (about 20 mg isocoumarin/100 g tissue; La Fuente and others 1996) (Figure 1). These results suggest that a stress or combination of stresses can be used to selectively target the increase of health-promoting compounds and avoid the accumulation of undesirable compounds.

The different responses observed in our study are related to the specific enzymes that were synthesized in the phenylpropanoid metabolism, including PAL and other enzymes of specific branch pathways. PAL catalyses the 1st committed step in the phenolic biosynthesis pathway, after which individual branch pathways make possible a range of phenylpropanoid secondary compounds (Schutte 1992). However, not all branches operate in a given tissue (Whetten and Sederoff 1995).

The identification of additional factors that may affect a stress response would also be a key component in designing strategies to increase nutraceuticals levels in tissues. For example, the presence of a stress signal, the stress intensity, a saturation response of the tissue (Saltveit 1997), produce maturity, produce cultivar, temperature, or the initial phenolic levels within tissues (Reay and Lancaster 2001) may be important factors in abiotic stress responses. Work with lettuce has shown that tissue with low initial levels of phenolic compounds had a higher phenolic accumulation in response to heat shock stress (Saltveit 2000). Similarly, apple tissues with low initial levels of anthocyanins have higher capacity to respond to UV-B light treatments for anthocyanin accumulation compared with tissues with higher initial levels (Reay and Lancaster 2001).

We found that treating whole purple-fleshed potatoes with methyl jasmonate would increase anthocyanin levels only in tubers with initial low anthocyanin concentrations (Figure 2). The induced anthocyanin observed was not related to ethylene production stimulated by methyl jasmonate, because ethylene treatments did not affect anthocyanin accumulation. These results may imply that there is a threshold level above which, certain secondary metabolites will not accumulate in the tissue; however, there is need to understand how this response is biochemically controlled.

Another factor to consider would be the potential use of selected chemicals to modulate the response of stresses in tissues. These selected chemical treatments may inhibit the action of PAL, polyphenol oxidase activity, or the oxidation of phenolic compounds.

Figure 1—Sliced purple carrots treated with methyl jasmonate and ethylene for 5 d at 20 °C induced accumulation of (a) total phenolics and (b) isocoumarin. Vertical bars represent SD (n = 3).

Figure 2—Total anthocyanins accumulated in whole purple potatoes treated with methyl jasmonate and ethylene after 10 to 14 d at 20 °C. Vertical bars represent SD (n = 3).
(Tomas-Barberan and others 1997, Peiser and others 1998). For example, the use of antioxidants, calcium solutions, and exclusion of oxygen may reduce the oxidation of stress synthesized phenolics with health promoting properties (Kang and Saltveit 2002). Other chemicals like 1-methylcyclopropene may be used to prevent ethylene-induced isocoumarin formation in carrots or the accumulation of phenolic compounds responsible for russet spotting in lettuce (Fan and Mattheis 2000). Potential uses of 1-methylcyclopropene have been recently reviewed (Blankenship and Dole 2003).

Increased nutraceutical content of fresh and processed products

Phenolic compounds are the major contributors to the antioxidant properties of fresh produce (Wang and others 1996; Prior and others 1998; Gil and others 2002) and are associated to antimutagenic and anticancer properties as well as to reduction of cardiovascular diseases (Dillard and German 2000).

Abiotic stresses such as wounding and phytohormones may lead to an increased phenolic concentration with health-promoting properties when applied to tissues (Figure 1 and 2). When wounding is applied to purple-flesh potato or orange carrot tissues, there is an increase in phenolic content and antioxidant capacity after 48 h (Figure 3). In sliced potatoes the increase in total phenolic content is approximately 76.7%, with a corresponding increase of antioxidant capacity of about 95.5%. For sliced carrots the increase in phenolic content is about 35.7%, whereas the antioxidant capacity increases by 16.6%. The phenolic compounds induced in both tissues differ in their specific antioxidant activities (defined as the ratio between antioxidant capacity and phenolic content of tissue). For example, the induced carrot phenolics have a higher specific antioxidant activity (about 1056 μg Trolox/mg phenolics) than induced potato phenolics (about 651.6 μg Trolox/mg phenolics).

Because the stress-induced phenolic compounds increase the antioxidant capacity of the tissues, we may consider stresses to be used as potential tools to render healthier products. The fresh produce industries, such as the fresh-cut industry, may benefit from wounding stress and possibly other stresses to obtain healthier fresh-cut products. Similarly, food processing industries that utilize wounding processes, such as in the production of potato chips or french fries, may benefit by obtaining healthier processed products.

The recommended daily intake of antioxidants for humans to obtain benefits is an estimated equivalent of about 3000 to 3600 μmol Trolox/day, from which fruit and vegetable consumption represents less than 50% or about 1200 to 1640 μmol Trolox/day (Prior and Cao 2000). Controlled stresses provide an opportunity to obtain products with higher antioxidant capacity that could meet the daily requirement.

Phytochemicals may have a diverse range of properties, in addition to nutraceutical use, that are very attractive to the industry, such as antimicrobial properties and color potential. For example, anthocyanins as colorants can cost approximately $1000/kg anthocyanin in the food market, making crops high in anthocyanin content good candidates for extraction operations of the colorant material. Thus, a potential use for abiotic stresses could be inducing the accumulation of specific compounds with the objective of enhancing the extractable yield. We have shown that stresses such as methyl jasmonate can induce the accumulation of anthocyanins in

![Figure 3—Slicing orange carrots and purple potatoes and storing for 2 d at 20 °C increased (a) the total phenolic content and (b) the antioxidant capacity of the tissue. Vertical bars represent SD (n = 3).](image)

![Figure 4—Proposed concept for using controlled postharvest abiotic stresses to enhance the nutraceutical content and adding value to fresh fruits and vegetables](image)
whole purple-fleshed potatoes increasing about 61% compared with a control (Figure 2). Methyl jasmonate also can be used in purple carrots to induce phenolic compounds for further extraction process without accumulating undesirable compounds like isocoumarins (Figure 1). Even more, selecting appropriate abiotic stresses and the appropriate target tissue may render extractable phenolic compounds with higher specific antioxidant properties as observed for carrots when compared with potatoes (Figures 3).

Proposed concept

In this paper we present a novel concept that is summarized in Figure 4. In this concept, controlled postharvest abiotic stresses, such as wounding, phytohormones, UV light, other radiations, altered atmospheric gas composition, and water stress, can be applied to fresh fruits and vegetables to induce the synthesis of specific active compounds and or decrease the synthesis of undesirable compounds. The targeted secondary metabolites would be related to an increased bioactive or functional property, such as increased antioxidant activity, antimicrobial properties, anticancer activity, or color potential. However, there is a need to understand how different stresses trigger the specific enzymes involved in the synthesis of the targeted compound, as well as the possible interaction between different stresses and the response of the tissue.

The controlled abiotic stresses can be used as tools by the fresh produce industry to create healthier products by enhancing the nutraceutical content of fresh-cut or whole products. Similarly, the controlled abiotic stresses can be utilized by the food processing and dietary supplement industry as tools to enhance the extractable yields of specific active compounds that have nutraceutical or other functional properties.

Some treatments, such as wounding, are already being used by the industry, whereas others such as UV radiation may be readily incorporated into the process line. However, as new stress treatments are characterized, there will be need for further studies to define their optimum incorporation into the processing line. For example, slicing potatoes and waiting for phenolic synthesis to occur may offer a challenge in finding ways to reduce the overall processing time. Other challenges to encounter may be the potential negative effects of increased phenolic content on browning potential, the altered taste of fruits and vegetables, such as increased astringency, or the appropriate criteria for selecting fruits to work with. However, as knowledge is gathered in this field, many of these challenges will be eventually solved. Ideally, the use of controlled stresses will be applied to redirect synthesis of secondary metabolites to obtain a specific targeted nutraceutical composition with low synthesis of undesirable compounds and avoiding producing quality changes.

This new research area may develop parallel to the trend observed in other fields dealing with nutraceutical research activities. For example, discoveries of novel bioactive and functional properties of specific compounds may create the need for developing technologies to increase nutraceutical levels in crops. Thus, controlled abiotic stresses may be a complementary or an alternative strategy to other approaches such as breeding programs, production management, or genetic engineering activities.

Conclusions

CONTROLLED POSTHARVEST ABIOTIC STRESSES CAN BE USED TO ENHANCE THE NUTRACEUTICAL LEVELS IN FRESH FRUITS AND VEGETABLES. However, there is need to understand how different crop tissues and their specific secondary metabolic pathways respond to different abiotic stresses, applied alone or in combination with others. This would be the basis for designing strategies to develop novel tools that will open the possibility of tailoring fresh produce with enhanced health benefit properties for use of the fresh produce and food processing industries.

References

Heredia J, Loaiza J, Cisneros-Zevallos L. 2001. Phenolic synthesis in maroon carrots treated with ethylene and methyl jasmonate stored at different tem-

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