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## Viewpoint

# Nonthermal processing technologies as elicitors to induce the biosynthesis and accumulation of nutraceuticals in plant foods



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## ABSTRACT

**Background:** Fruits and vegetables contain high levels of nutraceuticals, and thus their consumption is associated with the prevention of different chronic-diseases. The application of postharvest abiotic stresses (i.e. wounding stress) induces the accumulation of bioactive compounds in fresh produce. In this context, previous reports in literature suggest that nonthermal processing technologies [i.e. ultrasound (US), high pressure processing (HPP), and pulsed electric fields (PEF)] activate the biosynthesis of nutraceuticals in crops by a similar mechanism exerted by wounding stress. However, research on this area is scarce and it is still under debate if the higher levels of nutraceuticals detected in plant foods treated with emerging technologies are due to higher extractability or due to elicitation of metabolic pathways.

**Scope and approach:** In the present view-point paper, the response of horticultural crops to abiotic stresses with special emphasis to wounding is reviewed as a basis to propose a hypothetical model explaining how US, HPP and PEF technologies could act as abiotic elicitors for the biosynthesis of nutraceuticals.

**Key findings and conclusions:** Reports on literature strongly suggest that US, HPP and PEF induce immediate and late stress responses similar to wounding stress. However, further studies including “omic” approaches and physiological measurements, as response variables must be performed to validate the model herein proposed. Furthermore, additional investigations should elucidate optimum nonthermal technologies processing conditions that induce the highest accumulation of nutraceuticals in horticultural crops. These crops could be commercialized in the nutraceutical market or used as raw materials for the production of functional foods.

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## 1. Introduction

The application of postharvest abiotic stresses in horticultural crops has received great attention in recent years because they induce the biosynthesis and accumulation of bioactive compounds with health-promoting properties (Cisneros-Zevallos, 2003; Jacobo-Velázquez & Cisneros-Zevallos, 2012). In this context, recent reports indicate that emerging technologies such as ultrasound (US), high pressure processing (HPP) and pulsed electric

fields (PEF), when applied during postharvest, could also act as abiotic elicitors for the biosynthesis and accumulation of plant bioactive molecules (Ortega et al., 2013; Vallverdú-Queralt et al., 2013; Yu, Engeseth, & Feng, 2016; Cuéllar-Villarreal et al., 2016). However, research on this area is scarce and is still under debate if the higher levels of nutraceuticals detected in crops processed with emerging technologies are due to higher extractability or due to elicitation of metabolic pathways.

In order to better understand how emerging technologies elicit the biosynthesis of secondary metabolites additional studies reporting their effect in the stress physiology of plants are needed. There are few reports in literature supporting that oxidative stress produced after treatment with certain emerging technologies could be responsible of the accumulation of antioxidants (Schreck,

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Dörnenburg, & Knorr, 1996; Dörnenburg & Knorr, 1998; Lin, Wu, Ho, & Qi, 2001). Furthermore, US, HPP, and PEF induce cell membrane permeabilization (Knorr, 2003), feature that could trigger a stress response similar to that produced by wounding stress.

In the present view-point paper, the response of horticultural crops to abiotic stresses with special emphasis to wounding stress is reviewed as a basis to propose a hypothetical model explaining how US, HPP and PEF technologies could act as abiotic elicitors. Likewise, future perspectives on the investigation of nonthermal processing technologies as abiotic elicitors are discussed.

## 2. Physiology of stress response in horticultural crops: immediate and late stress responses

The responses of plant cells to abiotic stresses can be divided into an immediate and a late response. The immediate response starts with the production of primary and secondary stress signaling molecules, which activate signal transduction networks involved on the *de novo* synthesis of transcription factors that regulate gene expression of the plant secondary metabolism (Cisneros-Zevallos, Jacobo-Velázquez, Pech, & Koïwa, 2014). The late response occurs as a consequence of the immediate response and is associated with the production of biochemical constituents (i.e. enzymes and secondary metabolites) required for the acclimation of plants to abiotic stresses (Jacobo-Velázquez & Cisneros-Zevallos, 2012).

Regarding the immediate and late response of horticultural crops to wounding stress, leading to the accumulation of phenolic compounds, it has been reported that upon the application of wounding, ATP is released from damaged cells. This extracellular ATP serves as a primary wound signal that diffuses through intercellular spaces, until they reach and bind to ATP plasma membrane receptors of undamaged cells (Jacobo-Velázquez, Martínez-Hernández, Rodríguez, Cao, & Cisneros-Zevallos, 2011; Song, Steinebrunner, Wang, Stout, & Roux, 2006). ATP binding to its receptors elicits the production of secondary signaling molecules [i.e. reactive oxygen species (ROS), ethylene and jasmonic acid] (Jacobo-Velázquez, González-Agüero, & Cisneros-Zevallos, 2015; Song et al., 2006). These secondary signals diffuse through the cytosol, initiating the signal transduction network leading on the activation of transcription factors that trigger the biosynthesis of nutraceuticals as a late stress response (Cisneros-Zevallos et al., 2014; Jacobo-Velázquez et al., 2011). ROS and ethylene elicit the biosynthesis of phenolic compounds (Jacobo-Velázquez et al., 2015), whereas ethylene and jasmonic acid induces the biosynthesis of glucosinolates (Villarreal-García, Nair, Cisneros-Zevallos, & Jacobo-Velázquez, 2016). On the other hand, ROS is associated with the stress-induced biosynthesis of carotenoids (Bouvier, Backhaus, & Camara, 1998).

## 3. Ultrasound (US), high pressure processing (HPP) and pulsed electric fields (PEF) as abiotic elicitors

As earlier described, according to recent reports nonthermal processing technologies could act as abiotic elicitors for the biosynthesis of nutraceuticals in horticultural crops. This hypothesis was first formulated when different studies reported increases in the quantification of bioactive molecules immediately after processing or during storage of plant foods treated with emerging technologies. A summary of these studies including US, HPP, and PEF is shown in Table 1. Based on previous reports, the immediate and late stress responses induced by these technologies are reviewed in the following sections.

### 3.1. Ultrasound (US)

In the last decade, power US emerged as an alternative processing option to conventional thermal approaches for pasteurization and sterilization of food products (Rawson et al., 2011). Ultrasound alone or combined with mild thermal preservation techniques is receiving great interest since microbial inactivation can be achieved and phytochemical and organoleptic characteristics (texture, color, taste) of food can be conserved. Ultrasound has also been used in food processing to enhance operations such as filtration, defoaming, cooking, degassing, cutting, drying, meat tenderization, homogenization, and crystallization, among others (Chemat, Zill-e, & Khan, 2011).

Ultrasound is a form of energy generated by acoustic waves of frequencies higher than 20 kHz and can be propagated in gases, liquids or solid medium. Ultrasound can be divided into 2 groups: high and low intensities. High intensity treatments comprise low frequency US (between 20 and 100 kHz) with high energy, whereas low intensity treatments include high frequency (higher than 100 kHz) acoustic waves with low energy (Nowacka & Wedzik, 2016). Low intensity US is used in the medical field as a scanning and imaging tool. However, in the food industry it serves as a physical tool for quality assurance. Low intensity US can provide a rapid, accurate, inexpensive, simple, and non-destructive method to analyse the physicochemical properties of food and monitor the changes during food production processes (Chandrapala, Oliver, Kentish, & Ashokkumar, 2012; Nowacka & Wedzik, 2016). On the other hand, high intensity US is used for disrupting and breaking cellular structures, which later can activate or inhibit physicochemical alterations in food, leading to the intensification of heat and mass transfer based processes (Nowacka & Wedzik, 2016).

As a result of cell membrane damage produced by US, it has potential application for the extraction of nutraceuticals in plant foods (Baysal & Demirdoven, 2011). For instance, it has been stated that ultrasound-assisted extraction is an efficient process for phenolic compound extraction in carrot pomace and strawberries (Herrera & de Castro, 2005; Jaabar et al., 2014). Another investigation showed that US treated strawberries (40 and 59 kHz) had higher levels of vitamin C compared to the control and other US treatments (25 and 28 kHz) (Cao et al., 2010). A more recent investigation by Nowacka and Wedzik (2016) reported a substantial increase of carotenoid content in carrots that were treated with US. Authors found a 42.3%, 49.9% and 41.1% increase after 10, 20, and 30 min of ultrasonication at 35 kHz and 3 W/cm<sup>2</sup>.

The effectiveness of US as an extraction technique has been proven in other plant foods such as guava juice, where a significant increase of ascorbic acid was obtained after sonication, and when sonication was combined with carbonation this increase was greater (Rawson et al., 2011). Likewise, Sales and Resurreccion (2010) found that *trans*-resveratrol, *trans*-piceid, and *p*-coumaric-, caffeic-, ferulic-acids, and total phenolic content achieved their greatest extractability in peanuts when a combination of US and ultraviolet (UV) radiation treatment was applied, in comparison to the control and to US or UV alone.

It is important to point out that all these increases in the quantification of nutraceuticals detected after US treatments could not be attributed to biosynthesis. As described for wounding stress, the biosynthesis of secondary metabolites in stressed horticultural crops is observed as a late stress response. Therefore, the immediate increase in nutraceuticals detected after US treatments are related with an increase in the extractability of nutraceuticals due to cell membrane disruption (Nowacka & Wedzik, 2016). Furthermore, changes in the chemical structure of macromolecules, where

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