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Review and safety criteria in advanced oxidation processes in vegetables, mediated by hydroxyl radical.

Report		
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Objective :	Review and safety criteria in advanced oxidation processes in vegetables, mediated by hydroxyl radical.

Introduction

Ethylene is an invisible, colorless and odorless gas, which has no known dangerous effect in humans, on concentrations found within the storage chain and sale of fruits and vegetables. The ethylene molecule is relatively small and simple: it consists of two carbon atoms associated with four hydrogen atoms. The molecular weight of ethylene is 28.05 $gmol^{-1}$ and fruits and vegetables produce different amounts of ethylene as they mature[1]. Normally, ethylene cannot be detected by humans, although sometimes only experienced people can smell large amounts, but to this fact other volatile organic compounds of fruits and vegetables[2] also contribute. As its specific weight (1,178 kgm^{-3} to 15ºC) is similar to that of air (1,225 kgm^{-3} to 15ºC), ethylene freely diffuses to any other adjacent fruit or vegetables and to the spaces in which they are stored.

In addition to CO_2 and O_2, ethylene is the most important gas to be monitored and controlled in the fruit and vegetable supply chain[3,4]. Less than 1 part per million (ppm) in volume of ethylene gas, is enough to trigger the maturation process of the climatic fruit (which can continue to mature, once collected). Ethylene is considered a plant hormone that controls a wide range of physiological processes. During storage, after harvesting fruits and vegetables, ethylene may induce effects including senescence, over-maturation, accelerated quality loss, increased susceptibility to fruit pathogens and affecting different physiological processes. In addition to the endogenous production of ethylene by plant tissues, there are also external sources such as contaminants and the own metabolism of plants and fungi.

Concentration of ethylene

Knowing the concentration of ethylene in fruit and vegetable storage areas and the presence of harmful elements for products stored in cold rooms is a problem for production and consumer companies. Among the harmful elements are volatile organic compounds (VOCs), as a result of the metabolism of stored products the same, fungi, dispersed by the air through their spores and ethylene which, although encompassed in VOCs, deserves attention apart, given its character as a vegetable hormone, triggering the ripening processes in the fruits.

Solutions based on advanced oxidation processes (AOP) have been routinely used through photocatalysis, preferably with titanium dioxide (TiO_2) and ultraviolet (UV) radiation that, unlike those made with ozone (O_3), are safer by directly producing hydroxyl radicals (OH•) responsible for oxidation processes, preventing the accumulation of toxic gases[5,6].

OH• production occurs naturally in organisms as well as in the atmosphere. OH• is formed in biological systems by oxidative metabolism in which, predominantly in mitochondria, superoxide radicals (O_2-•) are formed as an unwanted byproduct. Superoxide radical can be removed by the enzyme superoxide dismutase. The product, H_2O_2, can be disposed of[12] by other enzymes (catalase), or may undergo a Fenton reaction in the presence of transitional metal ions to generate OH•.

In the oxidation of ethylene up to CO_2 and H_2O it is necessary a series of steps that produce intermediate products, which can be even worse than the original product. Intermediate products in the case of ethylene are ethylene oxide, formaldehyde, or methanol depending on the oxidative characteristics of the medium, all with hygienic hazards. This situation is complicated as the largest is the size of the molecule to be destroyed, increasing the chances of intermediate products. VOC's measurement allows you to determine whether the scrubber process reduces the levels present and whether secondary products resulting from oxidation appear.

In a study to determine the concentrations of ethylene, VOC's and fungal colony forming units (CFUs) in products stored in cold rooms exposed to AOP[7], the samples were analyzed using different methodologies and eventually analyzed by thermal desorption and gas chromatography with mass spectrometer (CGMS) for the identification of different compounds. All results showed a significant reduction in study variables. In the specific case of the chamber where melons were stored (Cucumis melo) the concentrations of ethylene before and after the AOP went from 1.5 - 3.4 ppm to 0.6 - 1.3 ppm respectively and in the chamber where peppers were stored (Capsicum annuum), the CFU went from an average of 149, AOP to 25 CFU's after treatment is applied. The choice of study parameters was determined, in the case of ethylene, by their importance in fruit aging processes limiting their duration in cold rooms; in the case of CFUs due to disease problems in stored fruits and VOC because they are generally compounds from the metabolism of stored products that produce the odors of stored products and because they can worsen the quality of these, being absorbed by other fruits or vegetables.

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The OH• radical is highly reactive and will react instantly with virtually any molecule it finds, reacting almost instantly at the site of its formation due to its high reactivity and short half-life. The distribution of an OH• radical attack depends on the density of electrons at the site of the attacked molecule. Due to its electrophilic nature, the OH• radical is preferably added to the site with the highest electron density. Other reactive oxygen species (ROS) that are not present in a radical form, such as O_3 cross biological membranes expanding their field of action and toxicity for longer periods of time[8,9].

Much of the VOC in the atmosphere comes from plant emissions, which is more evidence that the composition of the Earth's atmosphere is largely determined by biological activity. OH•/VOC's reactions lead to the formation of alkyl radicals (R•), alcoxi radicals (RO•), peroxi radicals (RO2•) and other species, which are transformed by decomposition, isomerization or hydrolysis, leading to the formation of oxygenated compounds, such as alcohols, carbonyls (aldehydes or ketones), carboxylic acids and hydroxyphyls[10]. Both OH• and O_3 contribute more or less equally to the oxidation of d-limonene[11].

Reactions involving OH•

There are 3 main types of reactions involving OH•: hydrogen abstraction, electron addition and transfer. All these reactions predict the effect caused by OH•. Basically, all reactions lead to the formation of new radicals and therefore propagate chain reactions. An example of abstraction is OH•'s reaction to alcohols. OH• extracts H• and forms water, leaving an unpaired electron in the alcohol carbon atom. Easily, OH• can be added to double bonds to form an oxygenated intermediate derivative that can also participate in electron transfer reactions[12]. In the case of an aromatic compound, OH• follows the mechanism of an SAE, during the process of its incorporation into the aromatic ring.

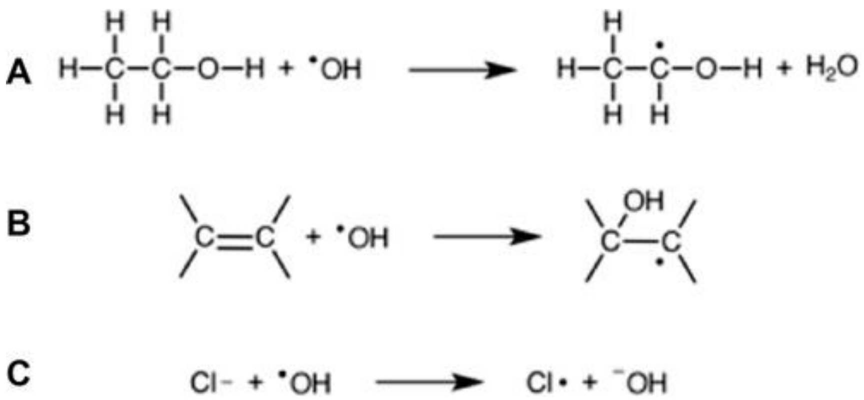

Image 1. Three main types of OH• reactions: (A) hydrogen abstraction; (B) addition to the double link; (C) electron transfer. Taken from Treml & Šmejka, 2016: Flavonoids as Potent Scavengers of Hydroxyl Radicals.

There is a difference between OH• getters and antioxidants. The mechanism of action of OH• removers is direct scanning, while antioxidants include OH• removers, transforming oxidation precursor compounds (such as O_3), ions involved in metal laning and increased activity and production of antioxidant enzymes. An imbalance of this process leads to a mismatch in the control of ROS, resulting in effects on plant cellular functionality. Thus, the production of

ethylene in tangerine epicarpium tissues with dark spots has been shown to be lower than in spotless tissues and these cells showed significant increases in membrane permeability, hydroxyl radical production and lipid peroxidation[13].

On the other hand, it is known that OH• can cause oxidation damage that leads to damage to the cell wall and deterioration of the quality of banana fruit during storage[14]. The metabolism of ROS may also depend on the action of ethylene15 and therefore influences the maturation and senescence and shelf life of the fruit. Ren et al.[16] suggest that improving the quality and prolonging life of mango fruits can be achieved by reducing oxidative damage caused by ROS during maturation. Other authors consider that, given the role of OH• in modifying cell wall polysaccharides[17], inhibition of OH• could contribute to maintaining the firmness of these fruits. On the other hand, it has also been proven, as the biosynthesis of flavonoids in plants is improved almost exclusively by oxidative stress[18], in fact, the reducing functions of flavonoids are of key importance in plants, under severe stress conditions.

Oxidative stress in vegetables

Plants exhibit greater synthesis of polyphenols such as phenolic acids and flavonoids under abiotic stress conditions, helping the plant cope with environmental constraints. An organism's exposure to unbalanced oxidative stress has many biological consequences. Oxidative stress mediated by free radicals ends up being beneficial to the plant, as it promotes the production of secondary metabolites and adaptation of the plant defense system thus contributing to a greater nutritional benefit. It has been suggested that a threshold level of free radicals is necessary for the normal physiological functioning of plants[19].

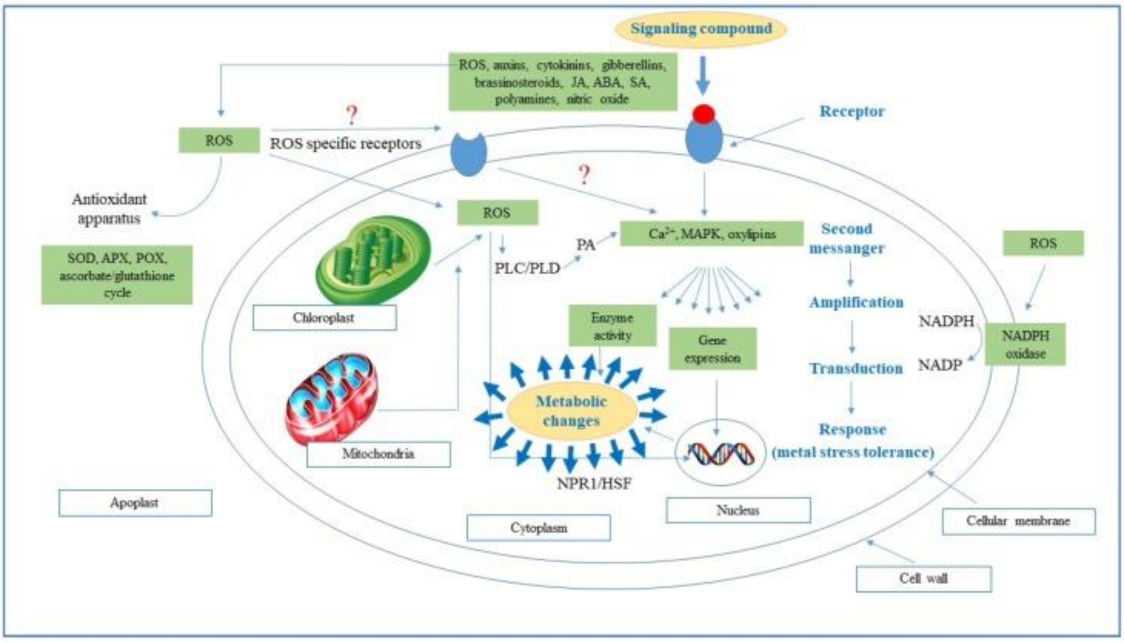

Image 2. Outlining the transmission and transduction of signals in plant cells. Abbreviation: ABA, abscisic acid; APX, ascorbate peroxidase; HSF, redox sensitive transcription factor; JA, jasmonic acid; MAPK, mitogen-activated protein kinase; NADP, oxidized dinucleotated adenine nicotinamide; NADPH, reduced

dinucleotated adenine nicotinamide; NPR1, redox sensitive transcription factor; OXI1, seine / threonine kinase; PA, phosphatidic acid; PLC / PLD, class C and D phospholips; POX, peroxidas; ROS, reactive oxygen species; SA, salicylic acid; SOD, superoxide dismutase. Taken from: Farooq et al.,2019

Flavonoids remove hydroxyl radicals (OH•) generated by UV photolysis of hydrogen peroxide[20]. Twenty-five vegetables (artichoke, asparagus, beetroot, beans, broccoli, Brussels sprouts, carrot, cauliflower, celery, chicory, cucumber, eggplant, escarole, garlic, green beans, leek, lettuce, corn, onion, pea, pepper, radish, spinach, chard and zucchini) were used to evaluate their antioxidant activity. All the fresh vegetables studied were able to remove the radicals lipoperoxyl and hydroxyl. All vegetables also had a good antioxidant capacity, except cucumber, endibia, carrot and zucchini. Vegetables stored (7 days) in a household refrigerator recorded the same antioxidant activity as fresh samples, except cucumber and zucchini (lipid peroxidation) and broccoli, Brussels sprouts and leek. Canned vegetables showed a more pronounced loss of antioxidant activity than frozen vegetables compared to fresh vegetables. Over the life of processed vegetables (8 months for frozen vegetables and 18 months for canned vegetables), some products showed losses (19-48%) lipoperxyl radical removal ability and total antioxidant activity[21].

Plants that grow in stressful environments have the ability to biosynthesize more phenolic compounds compared to plants that grow under normal conditions.

These compounds have antioxidant properties and are able to eliminate free radicals, resulting in reduced cell membrane peroxidation[22].

Flavonoids can improve the process of metal processing, which helps reduce levels of harmful hydroxyl radicals in plant cells[23.24] and this fits with the observation that flavonoid levels in plants have been increased by excess metals[25]. Low metal toxicity, the accumulation of specific flavonoids that are involved in assisting the plant's defense mechanism also increases, including anthocyanins and flavonals.

Based on the results of this review, we can suggest that the preservation of fruits and vegetables in cold rooms is dependent on the accumulation of VOC, the proliferation of microorganisms, the conditions of the chambers and the characteristics of each vegetable. The elimination of VOCs (including ethylene) can be used as an indicator of the operation of oxidizing processes as environmental scrubbers of the storage and storage chambers.

The previously determined behavior of the Wadu02 device, it allows to affirm that the radical OH• is the oxidizing element responsible for the AOP used and, consequently, presents a potential action on the modulation of ethylene levels that favourably affect fruits and vegetables, as well as its microbiological control.

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