Application of Molecular Hydrogen to Postharvest Produce

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Molecular hydrogen (H_2) has been found to have significant effects in a range of organisms, from plants to humans. In the biomedical arena it has been found to have positive effects for neurodegenerative disease and even for treatment of COVID-19. In plants H_2 has been found to improve seed germination, foliar growth, and crops: effects being most pronounced under stress conditions. It has also been found that treatment with H_2 can improve the postharvest preservation of fruits, vegetables and flowers.

Keywords: hydrogen-rich water ; flowers ; fruit ; molecular hydrogen ; postharvest ; vegetables

1. Introduction

Molecular hydrogen (H_2) is becoming recognized as a molecule that has significant effects on biological systems [1]. In the medical arena H_2 treatment has been suggested for a range of conditions [2], including neurodegenerative conditions [3][4] and COVID-19 [5][6]. It has been used for decades in deep sea diving, with H_2 concentrations being used at 49% (with 50% Helium and 1% oxygen, in a mixture called Hydreliox). H_2 leaves no by-product residues if used as a gas. There are no regulatory issues that are known in respect of the food industry, so this all suggests that H_2 is safe for human consumption, and therefore its use on food products should also be safe. Furthermore, H_2 is colorless, odorless and tasteless, so H_2 makes an ideal treatment for food processing.

Food waste is a major challenge undermining food security and income generation in many countries around the world. The United Nations Sustainable Development Goals aim to half per capita food waste by 2030 [7]. Despite this target, the amount of food waste produced globally is increasing [8]. Postharvest food waste has significant nutritional, environmental, as well as financial impacts for producers and consumers. Thus, by preventing waste at the different stages of food supply chain, we would be able to increase the availability of food without requiring additional resources and adding extra burden on the environment.

Therefore, developing new methodologies to prevent or reduce food waste is of major importance, especially because the world population is estimated to reach approximately 10 billion by 2050, which will require an increase of at least 70% in food production [9]).

There is good evidence that H_2 has potential uses in agriculture, as previously reviewed [10][11][12], with effects on seed germination, plant growth and crop yields. It has been suggested that H_2 treatments may be adopted as part of agricultural practices and has already been used in field trials with rice [13], for example.

Here, the evidence that H_2 can be used for the prevention of spoilage of food products, and therefore in their storage and transportation, will be reviewed. Furthermore, the current aspects of the mechanisms of action will be briefly visited.

2. Application of H_2 to Produce

There are several methods for the application of H_2 to food products. The easiest way would be to use H_2 as a gas. An example of this treatment is Hu et al. [14], in the treatment of kiwifruit. H_2 can be readily commercially purchased, or it can be produced locally by the electro-hydrolysis of water—which also produces O_2. Any O_2 generated can be separated or alternatively used as a H_2/O_2 gas mixture, referred to as oxy-hydrogen (HHO: 66% H_2/33% O_2) [5]. However, there are safety issues to be considered if H_2 is being used in the gaseous form, as exemplified by the disastrous explosion of the *Hindenburg* airship [15]. Therefore, caution needs to be exercised if H_2 gas is used in a confined space.

Alternatively, H_2 may be used in the form of a gas enriched solution, often referred to as hydrogen-rich water (HRW). This is widely used, an example being the work on heat stressed cucumber where both photosynthesis and the antioxidant capacity of the plants was altered [16]. Although probably of limited use in the treatment of food, a variation of this is the bubbling of a saline solution with hydrogen gas, to make hydrogen-rich saline (HRS), as used in a study to investigation hydrogen and radiation effects in mice cells [17]. HRW can be sprayed on foliage, or added to feed water, being poured straight onto the soil or growth medium. It is therefore relatively inexpensive and simple to use.

A more advanced variation on HRW is hydrogen nanobubble water (HNW). One of the issues with HRW (and HRS by extension) is that the H_2 will rapidly return to the atmosphere, so depleting the solution of H_2. This means that HRW needs to be used as a fresh solution. It also means that the biological material will be exposed to a bolus effect, where there is a very high concentration on treatment that does not persist. It also means that it is often hard to know the exact concentration of H_2 that the material gas is exposed to. To some extent the use of HNW should mitigate some of these issues, as it is reported that the solution has a higher concentration of H_2 which remains in solution for longer. An example of its use is a study on how hydrogen affects the copper toxicity of *Daphnia magna* [18].

H_2 can also be supplied to biological materials in the form of donor molecules, such as magnesium hydride (MgH_2). This was used in the study of the vase life of carnations, for example [19]. Donor molecules are likely to supply H_2 gas to a biological material more slowly and over a longer period of time, so perhaps mitigating the need for repeated applications, which may be needed with HRW. Nanoparticles have been suggested for gas delivery, for example with nitric oxide (NO) [20]. Similar technologies are being developed for the storage and delivery of H_2 as well [21]. However, donors often give by-products that may be detrimental to human health. If that is the case, then the use of such donors for the food industry would not be possible.

However, the application of H_2, regardless of the method, needs to have a thorough cost/benefit analysis, unless the need is to preserve food for sustaining a population, where cost may be of less consequence. If the amount (value) of food gained from treatment does not outweigh the cost of that treatment it is financially unsustainable. Having said that, the use of hydrogen has been mooted for a variety of industries, not least in supplying energy for methods of travel, be that car, train or airplane [22]. Therefore, there is a need for more cost-effective production and supply of H_2 gas, and this will no doubt bring the cost of its use down. This would be of great benefit for the use of H_2 in the food industry.

3. Application of H_2 to Postharvest Produce

As outlined above, there are various ways in which plant materials can be treated with molecular hydrogen. What can be gleaned from below is that treatment of fruit and vegetables has been relatively widely studied, using a range of plant species (**Table 1**).

Table 1. Some of the examples of molecular hydrogen treatment of postharvest fruit and vegetables. HRW: hydrogen-rich water; NO: nitric oxide; PPO: polyphenol oxidase; ROS: reactive oxygen species; SAEW: slightly acidic electrolyzed water.

Biological Material Treated	Manner of Treatment	Effects Seen/Comments	Reference(s)
Kiwifruit	HRW	Delayed ripening and senescence	[23]
Kiwifruit	H_2 fumigation	Better fruit, mediated by ethylene metabolism	[14]
Kiwifruit	(HRW) (and with slightly acidic electrolyzed water (SAEW))	Reduced loss of antioxidants such as flavonoids, and delayed chlorophyll loss. Reduced oxidative stress markers.	[24]
Rosa sterilis	HRW	Better fruit, mediated by ROS and energy metabolism	[25]

Biological Material Treated	Manner of Treatment	Effects Seen/Comments	Reference(s)
Okras	HRW	Delayed fruit softening, better cell wall maintenance	[26]
Litchi (Lychee)	HRW	Reduced pericarp browning, lower oxidative stress indicators	[27]
Chinese water chestnut	HRW	Less tissue yellowing, reduced oxidative stress, effects on the phenylpropanoid pathway	[28]
Tomato	HRW or H_2 fumigation	Reduced nitrite accumulation, with relevant enzymes affected	[29]
Banana	HRW	Delayed ripening, effects mediated by ethylene metabolism	[30]
Tomato	HRW	Altered defense responses, increased polyphenol oxidase (PPO) activity and NO	[31]
Strawberry	Gas in packaging	Better storage, lower fruit oxidation	[32]
Hypsizygus marmoreus	HRW	Better storage mediated by antioxidants	[33]

Hu et al. [23] treated kiwifruit with HRW and found that ripening was delayed and that the fruits stayed firmer for longer, with 80% HRW having the best results. Pectin solubilization was lower in the fruit and there was less oxidative stress in the cells, therefore less lipid peroxidation, whilst antioxidant superoxide dismutase (SOD) activity was increased. The inner mitochondrial membrane also maintained a better integrity. In a more recent paper H_2 was used as a fumigation treatment of kiwifruits [14]. This increased endogenous H_2 and delayed fruit softening. As with bananas [30], H_2 effects were found to be mediated by ethylene metabolism, with ethylene synthesis being inhibited by H_2, and concomitant decreases in 1-aminocyclopropene-1-carboxylate (ACC) [14]. Enzymes involved in this metabolism were appropriately altered too, i.e., ACC synthase and ACC oxidase. Zhao et al. [24] also studied kiwifruit and found that treatment with HRW significantly delayed the increase in soluble solid content, weight loss, and the total microbial load of the samples when compared with the controls. It also allowed the maintenance of chlorophyll, color, and firmness and improved the levels of ascorbic acid, total phenols and flavonoids during refrigerated storage. Interestingly, the authors also found that the aforementioned positive effects were even more pronounced when HRW was combined with a slightly electrolyzed water treatment.

Further studies into using H_2 gas as a fresh food preservative demonstrated that incorporating 4% of reducing hydrogen gas into the packaging (RAP) of strawberries can protect the color, texture, and nutritional parameters of the fruits when compared with conventional, modified atmospheric packaging (MAP). The results describe that through the addition of H_2 into the packaging headspace, oxidation of fruits is diminished, thereby preserving anthocyanin and phenolic content of strawberries. RAP also extended both the best before and expiration date periods longer than MAP. RAP could be considered as a green and non-toxic technique for preserving fresh fruits, helping producers, processors, and exporters to preserve and store strawberries for extended periods [32].

Rosa sterilis is economically important in Southwestern China [34]. HRW was used to treat the fruit and it was found that it reduced fruit weight loss, decay index and oxidative stress (both H_2O_2 and superoxide anions were reduced, as was malondialdehyde content) in the plant tissues [25]. Glutathione and ascorbate levels were increased, as were the activity of antioxidant enzymes, such as catalase (CAT) and SOD. Energy metabolism was also affected by H_2 treatments. Both the activities and gene expression of some key proteins were increased, including H^+-ATPase, succinate dehydrogenase and cytochrome oxidase (Complex IV). This increased both ADP and ATP levels, but reduced AMP.

HRW also delayed postharvest fruit softening in okras (*Abelmoschus esculentus* L.) [26]. Here, HRW improved cell wall biosynthesis, with higher pectin, hemicellulose and cellulose observed, particularly during the early phases of storage, and later in storage. Several genes which are involved in cell degradation were shown to have lower expression on HRW treatment. These were *AePME* (pectin methylesterase), *AeGAL* (β-galactosidase) and *AeCX* (cellulase). HRW treatment of litchi (lychee) before storage also maintained fruit quality [27], where pericarp browning was reduced, and total soluble solids maintained. This was mediated by HRW lowering oxidative stress within tissues, as seen with increased levels of reduced molecules (e.g., reduced glutathione (GSH)) and higher activity of antioxidant enzymes, such as CAT. HRW also maintained the color of Chinese water chestnut [28]. Here, yellowing of the tissues was delayed, and again oxidative stress characteristics were reduced, i.e., reduced ROS and higher antioxidant capacity. Flavonoids were less accumulated, with the effects reported to be due to the reduced action of the phenylpropanoid pathway.

In tomatoes, the treatment of fruits with either HRW or using H_2 fumigation resulted in less nitrite accumulation [29]. The enzymes involved in nitrogen metabolism were affected and nitrate reductase (NR) activity was decreased, whilst the activity of the enzyme responsible for nitrite reduction, i.e., nitrite reductase (NiR) was raised. Fumigation with just H_2 shows that the effects were specific to H_2, as nitrogen (N_2) and argon (Ar) were used as controls. This is particularly important for the discussion here, as nitrite is harmful to human health and much of the dietary nitrite comes from fruit and vegetables. To the best of the researchers' knowledge the effect of H_2 on nitrite accumulation during storage has been studied only for tomatoes. Further studies on other types of produce would help to clearly understand the underlying mechanisms and assess the potential of this method to be used for nitrite content reduction. Therefore, H_2 can not only maintain the fruit for storage, but potentially help retain nutritional value.

Very recently it was shown that HRW delayed the ripening in banana [30]. The color changes in the fruits were delayed, as were the degradation of cells walls and starch. The effects appeared to the mediated by ethylene. In ripening bananas, a rapid increase in ethylene synthesis upon maturity precedes an inordinate elevation in respiration and subsequent aging [35].

On a slightly different note, it was reported that H_2 altered the defense responses to *Botrytis cinerea* in tomatoes [31] where both 50% and 75% HRW had an effect. Polyphenol oxidase (PPO) activity was increased by HRW as was the content of nitric oxide, which together helped to increase the plant tissue’s pathogen defense.

Chen et al. [33] showed the effects of HRW postharvest (12 days at 4 °C) in a fungus, i.e., *Hypsizygus marmoreus.* 25% HRW was the best treatment used, with reduced electrolyte leakage and lower oxidative stress, as seen with reduced malonaldehyde content. As seen with higher plants, the antioxidant capacity of the fungus was increased. The gene expression and activity of key enzymes such as CAT, SOD and ascorbate peroxidase (APX) were increased.

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