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Review

Molecular Hydrogen: The Postharvest Use in Fruits, Vegetables and the Floriculture Industry

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Featured Application: It is proposed here that the use of molecular hydrogen should be considered more widely for the treatment of post-harvest fruits, vegetables and flowers. This can be applied as a gas, or in solution, and costs associated with its use expected to fall as hydrogen is adopted by other industries.

Abstract:

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. Therefore, H_2-based treatments may be useful for the storage and transport of food products. H_2 can be delivered in a range of manners, from the use of the gas to creating H_2-enriched solutions, such as hydrogen-rich water (HRW) or hydrogen nanobubble water (HNW). The exact action of H_2 at a biochemical level has yet to be established. Despite this, H_2 appears to be safe. Treatments of food with H_2 would leave no harmful residues, and H_2 itself is safe to use, as exemplified by its biomedical use. With H_2 production and transport being developed for other industries, H_2 is likely to become cheaper and its use for postharvest maintenance of food may be beneficial to explore further.

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

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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 we are aware of 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 [10-12], 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]
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 diminshed, 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 our 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. The authors conclude the abstract by saying "This study supplies a new and simple method to maintain the quality and extend the shelf life of mushrooms", which appears to the summation of this section of this review as H_2 is clearly adaptable and could be applied to range to postharvest plant materials.

4. Use of H_2 for the Floriculture Industry

As with fruits and vegetables, hydrogen treatment can be applied to flowers postharvest, therefore such treatments may be of interest to the floriculture industry. A range of flowers and treatments have been studied, as exemplified by the data in Table 2.

Table 2: Examples of molecular hydrogen treatment of flowers. ATP: adenosine triphosphate; HRW: hydrogen-rich water; HNW: hydrogen nanobubble water: H_2S: hydrogen sulfide; MgH_2: magnesium hydride; NO: nitric oxide.

Flowers treated	**Manner of treatment**	**Effects seen/comments**	**Reference(s)**
Lily (*Lilium* spp.) and Rose (*Rosa hybrid* L.)	HRW	Better vase life, increased antioxidants	[36]
Carnation (*Dianthus caryophyllus*)	HRW	Details not known	[37]
Carnation (*Dianthus caryophyllus*)	MgH_2 as a donor	Better flower life, mediated by H_2S and altered gene expression	[19]
Carnation (*Dianthus caryophyllus*)	HNW	Prolonged flower life, lower oxidative stress and senescence-enzyme activities	[38]
Lisianthus (*Eustoma grandiflorum*)	HRW	Better flower maintenance mediated by redox status	[39]
Lily (*Lilium* "Manissa")	HRW	Beneficial effects, mediated by NO and ATP synthase	[40]

Flowers are relatively easy to treat, once cut. HRW or HNW can simply be added to the feedwater in the vase, or the atmosphere can be gassed if flowers are being kept in a closed container. Donor molecules can also be used with relative safety as long as the flowers are not for human consumption.

The global market for cut flowers was approximately $34,347 million in 2019 [41], and it has been estimated that it will increase to $49,000 million by 2028. It has been reported that "flowers are vulnerable to large post-harvest losses" and therefore there "arises the need for the appropriate post-harvest handling technologies" [42]. The market for flowers demands that they look good and last for a long period once purchased. There is, therefore, a need for flowers to be maintained in good condition for transport, storage, and in their final place of display. Even in a domestic setting it would be possible for individuals to treat feedwater with donors or treatments which release H_2 into solution, such as those based in magnesium. The question that needs to be asked is: Is there any real benefit in using H_2 treatments?

Ren *et al*. [36] found that the use of HRW, at concentrations of 0.5% and 1%, increased the vase life and flower diameter of cut lilies (*Lilium* spp.). They also found that HRW (50%) had a similar effect in rose (*Rosa hybrid* L.). The content of leaf malondialdehyde in the leaves of lily decreased, which is a sign of lowered oxidative/nitrosative stress, and this data matched the fact that HRW increased the activity of antioxidant enzymes in both lily and rose. Cai *et al*. [37] studied the effects of HRW on carnations (*Dianthus caryophyllus*), with similar studies being more recently reported [19,38]. These latter reports used magnesium hydride as a donor or HNW, respectively. In the first of these studies, the use of MgH_2 could be enhanced by the inclusion of citrate to the buffer, prolonging the release of H_2 to the flowers. Under such treatments the flowers had a prolonged shelf life. Hydrogen sulfide (H_2S), a known signaling molecule in plants [43] (and animals [44]) was shown to increase in the plants, and a H_2S scavenger, hypotaurine, reversed the effects. Further, it was said that the redox status of the cells was reestablished, and the gene expression of *DcbGal* and *DcGST1*, both associated with senescence, was reduced. Overall, the authors concluded that the MgH_2/citrate mix had a positive effect on the flowers and the mechanism was mediated by H_2S signaling. In the second study [38], HNW was used, which the authors said prolonged the time that the H_2 was dissolved in the water and therefore gave longer treatment times. It was found that 5% HNW significantly increased the vase life of the flowers. This treatment lowered the activity of nucleases and proteases in the plant tissues, which also showed lower ROS and less oxidative stress.

Su et al. [39] looked at lisianthus (*Eustoma grandiflorum*) and concluded that the treatment with H_2 maintained the flowers, with the effects mediated by the maintenance of the cellular redox status. 2,6-dichlorophenolindophenol (DCPIP) was used as a possible H_2 inhibitor, although DCPIP is a relatively generic redox acceptor; it is commonly used in redox experiments. The authors comment that upon H_2 treatment, the flower tissues had reduced lipid peroxidation and increased activity of a range of antioxidant enzymes, such as CAT, SOD and APX. Finally, Huo et al. [40] looked at the effects of HRW in relation to the action of NO in lily (*Lilium* "Manissa"). Both extended the vase life of the flowers, but the effects of H_2 were reduced by NO inhibitors, suggesting a link. The authors used proteomic analysis of the downstream effects of HRW and NO, and then highlighted the protein ATP synthase CF1 alpha subunit (chloroplast) (AtpA) as being of particular significance. The expression of this protein was upregulated by HRW. The study concluded that HRW was beneficial for flower vase life, but that downstream of HRW may be NO metabolism and the ATP synthase complex.

It is clear, therefore, that H_2 treatments of flowers are beneficial, easy to use and safe. However, none of the reports regarding H_2 treatments in this arena discuss the cost/benefit of these treatments, whilst it is unlikely they will be large-scale adoption unless there is a tangible financial benefit.

5. Biochemical Effects of H_2

From the above it can be seen that H_2 has several effects and promises to be a suitable treatment for fruit, vegetables and flowers postharvest. However, the direct molecular action of H_2 remains somewhat controversial. This has previously been discussed [45], so a brief résumé will be given here.

In several of the papers above, a change in the antioxidant capacity of the plant material has been noted. This is, perhaps, a secondary effect, as there is little evidence that H_2 directly interacts with the antioxidant mechanisms of the cells, or with the cell signaling components that may lead to increased gene expression, which would underpin the increase in antioxidant enzymes, either their increase in protein or activity. Therefore, an alternate direct H_2 target needs to be found to account for how H_2 may reduce oxidative stress in plant cells.

It has been suggested that H_2 acts as a direct antioxidant, interacting with, and scavenging, both reactive oxygen species (ROS) and reactive nitrogen species (RNS) [46,47]. The defined targets were said to be the hydroxyl radical ($^{\cdot}$OH) and peroxynitrite (ONOO^{-}), both which have been reported as signaling molecules [48,49]. However, on the basis of the kinetic reactions between H_2 and these relatively reactive molecules, the physiological relevance of such interactions has been questioned [50], with the authors' conclusions stating: "H_2 does not prevent or repair oxidative damage by either HO$^{\cdot}$ or ONOOH." However, a possible mechanism for how H_2 may catalyze such reactions has very recently been mooted by others [51]. Here, H_2 is thought to interact with the iron (Fe) in the heme of hemoglobin, allowing the formation of hydrogen radicals and further downstream reactions. Whether this is acting under physiological conditions and how widely the mechanism may be extrapolated needs to be established. For instance, plant cells have no true hemoglobin, so could plant homologues [52] also partake in this reaction? Furthermore, there is a range of heme-containing enzymes which may directly interact with H_2 if such a mechanism is found to be common. These include instrumental signaling enzymes such as nitric oxide synthase (NOS) and guanylyl cyclase (GC), as well as proteins which may produce ROS and RNS signaling through electron leakage, such as the cytochromes in mitochondria and chloroplasts. Therefore, much more work needs to be carried out if we are to understand the ramifications of such H_2 action, or to rule it out.

H_2 is a relatively reducing molecule, and it may be acting through its redox potential [53], perhaps directly reducing the redox centers of prosthetic groups, such as cytochromes. There is a precedence for this in bacterial systems [54], and it might not be much of a stretch to posit such action in the cells of higher organisms, such as plants.

Alternative mechanisms may include an interaction of molecules with H_2 through the altered spin states of the H_2 molecule [55]. There is no experimental evidence for this, but it seems as though it is theoretically possible. What is unlikely is the direct modification of proteins by H_2 in a classical manner. There are numerous post-translational modifications of proteins, with some common adaptations involving small gaseous molecules and other related reactive molecules, including oxidation [56], *S*-nitrosylation [57], tyrosine nitration [58] or persulfidation [59]. H_2 is relatively unreactive and is not perceived to partake in synonymous reactions. Clearly, understanding the direct action of H_2 on biomolecules needs to be a priority for future research if the use of H_2 in industries such as food processing is to become customary. Although there are no known toxic effects of H_2 in humans, after all, it has been used in the diving industry for years [60], the actual biological actions of H_2 on plant products, which will be eaten, and long-term effects of human health would have reassurance if the molecular action(s) of H_2 were known.

6. Conclusions and Future Perspectives

H_2 has been found to have profound effects in plants, this includes the use of H_2 in postharvest processing and packaging of food products. This review has shown that H_2 can improve plant tissue tolerance against different abiotic/biotic stresses, regulate plant growth, increase antioxidant, extend shelf life, and decrease nitrite accumulation during the storage of produce.

H_2 may even be useful preharvest [61], so further work in this arena seems certainly worthwhile.

Therefore, if the cost/benefit analysis shows that there are pragmatic advantages to the use of H_2, it should be considered widely in the food industry. As H_2 is being dubbed "the fuel for 21st century" [22], it is likely that the production and transport of H_2 will be more widely adopted, and this would bring the cost down for the use of H_2 in the food industry.

However, there are a lot of questions which still need to be answered:

- Which food products would benefit from these treatments?

- How widely can these treatments be adopted by related industries, such as floriculture?
- Do we need to be concerned about safety: the gas is explosive, and donors leave by-products which may be toxic?
- What is the biochemical action of H_2 in plants? Are there any negative effects being induced?
- How deeply into plant tissues do H_2 treatments penetrate? Or are surface effects sufficient for the postharvest effects required?
- How long do the treatments last, and are repeated treatments required?
- Will cost benefit really make these treatments pragmatic?
- If transport moves over to H_2 as fuel, can this be incorporated into the storage of plant materials as the H_2 is being carried anyway?
- Can H_2 treatment be used to decrease the levels of foodborne pathogens on fruit and vegetables?
- Can H_2 be used in conjunction with current industry practices to increase effectiveness and facilitate adoption?

Having posed all these questions, and considered the evidence, it is clear that H_2 will have uses in the postharvest management of many food products, and it is certainly worth future investigation and considering for wider adoption as a preservative agent.

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