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Molecular hydrogen: a sustainable strategy for agricultural and food production challenges

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The world is confronting numerous challenges, including global warming, health epidemics, and population growth, each presenting significant threats to the stability and sustainability of our planet's ecosystems. Such issues have collectively contributed to a reduction in agricultural productivity, corresponding with an increase in demand and costs of essential commodities. This critical situation requires more sustainable environmental, social, and technological solutions. Molecular hydrogen (H₂) has been suggested as a "green" solution for our energy needs and many health, agricultural, and food applications. H₂ supplementation in agriculture may represent a novel and low-carbon biotechnological strategy applicable to the abundant production of crops, vegetables, and fruits in agri-food chains. H₂ is a potential green alternative to conventional chemical fertilizers. The use of a hydrogen-rich water irrigation system may also provide other health-related advantages, i.e., decreasing the heavy metal accumulation in crops. By adopting a H₂ strategy, crop producers, food processors, and decision-makers can contribute to sustainable solutions in the face of global challenges such as climate change, communicable disease epidemics, and a growing population. The versatile applications of H₂ in agriculture and the wider food industry position it as a uniquely suitable approach to address today's significant challenges, potentially fostering better crop production and positively impacting the agri-food chain. The present review is timely in combining the latest knowledge about the potential applications of H₂ in the agriculture and food industry, from farm to fork.

KEYWORDS

crops, foods, food security, green strategy, hydrogen-rich water, molecular hydrogen, sustainable technology

1 Introduction

Given the perishable nature of crops, fruits, and vegetables, these natural foods experience significant quality losses during postharvest storage and distribution (Singh et al., 2022). It has been reported that more than 1.3 billion tons of food go wasted every year, with the spoilage of fruits and vegetables accounting for almost 40% of these losses (Bhatnagar et al., 2022). Therefore, storing fresh produce presents a significant challenge due to the rapidly occurring physiological changes and susceptibility to microbial contamination and spoilage. Various postharvest methods of preservation, encompassing chemical treatments, application of ozone, controlled atmospheres, and innovative packaging materials, including antimicrobial packaging, coatings, and waxes, are under consideration or in use (Ali et al., 2023). Other methods, including non-thermal technologies, e.g., cold plasma and high-pressure processing (Pasquali et al., 2016; Nasri et al., 2023), are being explored to address postharvest losses. One of the methodologies gaining recognition and importance for preserving food quality is the application of H₂.

H₂ is a colorless, odorless, and simple diatomic gas composed of two hydrogen atoms. It has emerged as a compelling subject of investigation across diverse scientific disciplines, including energy research, space exploration, climate change, geology, microbiology, biomedicine, agriculture, and food technology (Figure 1). Research on the biomedical and agricultural use of H₂ is obscured by the copious research on its applications in the green energy sector. However, it is noteworthy that the research on the biomedical and agricultural benefits of H₂ has grown exponentially over the past 16 years (LeBaron et al., 2024).

Food security is a major challenge of the present time. Sustainable technologies that improve crop and food production, and storage must be a priority for the future. This is not the first instance H₂ has been proposed as a green alternative to chemical-based agricultural systems (Zulfiqar et al., 2021; Alwazeer and Çiğdem, 2022). However, it is a rapidly advancing field, particularly in China and Japan, making it timely to review the literature and discuss relevant issues. Although numerous reports have explored the potential uses of H₂ in agriculture, there is a lack of

literature on the potential uses of H₂ in food products and technologies.

The novelty of this review lies in exploring H₂ as a multifaceted and sustainable solution to contemporary challenges in food and agriculture. The specific aims and objectives of this study are:

Background/Hydrogen Research: With a view to providing a comprehensive overview of hydrogen (H₂) research, highlighting its emerging roles across various sectors, we review the historical development of H₂ research, current advancements, and key areas where hydrogen is becoming influential.

Hydrogen in Agriculture: Here, the application of hydrogen in agricultural practices is explored and the potential of H₂ for enhancing crop yield, improving soil health, and reducing the environmental footprint of agricultural practices is evaluated.

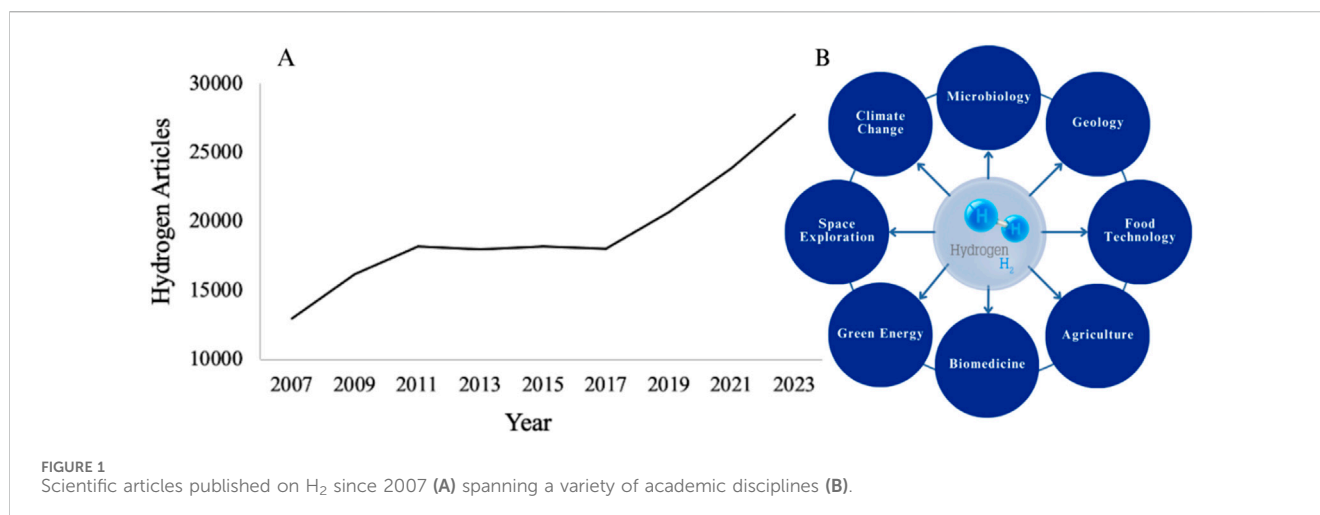
Hydrogen in Food Processing and Packaging: Assessment of the utilization of hydrogen in food processing and packaging industries allows us to analyze how H₂ could improve food preservation, extend shelf life, and ensure food safety during processing and packaging.

Hydrogen in the Valorisation of Food Waste: Investigating the role of hydrogen in the valorisation of food waste and exploring the methods by which H₂ can be used to convert food waste into valuable products, could help us identify a valuable resource for ensuring waste reduction and improving sustainability in the food industry.

The Bioactivity of Hydrogen: By reviewing existing empirical and theoretical studies on the effects of H₂ in biological systems we examine the bioactivity of hydrogen and its potential benefits for increasing harvest yield and preserving foods.

Regulations, Toxicity, and Safety of Hydrogen: Here we provide an overview of current regulations, assessing the safety profile of H₂, and identifying any potential risks in its application, highlighting toxicity, and safety considerations associated with hydrogen.

The Current Status of Research and Future Perspectives: To identify gaps in the existing literature, propose future research opportunities, and predict the potential trajectory of hydrogen applications across various sectors, we summarize the current state of hydrogen research and costs, and discuss future directions.



2 Hydrogen research

2.1 H₂ and health

In 2007, it was reported that hydrogen gas exhibited selective antioxidant properties in a rat cerebral infarction model (Ohsawa et al., 2007). In this study, a 2% hydrogen gas concentration effectively suppressed brain damage induced by the ischemia/reperfusion injury. The researchers also found that dissolving the H₂ gas into cell culture media could selectively reduce strong oxidants such as hydroxyl radicals ($\cdot\text{OH}$) and peroxynitrite (ONOO^-). However, H₂ did not react with biologically important reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2), nitric oxide (NO^\bullet), or superoxide ($\text{O}_2^{\bullet-}$) (Ohsawa et al., 2007), indicating that H₂ has selective antioxidant capabilities and may have important medical applications. Moreover, the high bioavailability of this gaseous molecule makes it an attractive candidate for its therapeutic actions. Hydrogen can easily diffuse through cell membranes and reach subcellular organelles due to its small and non-polar nature (Alwazeer et al., 2021).

There are now over 2,000 scientific publications, including 140 human studies, on the biomedical effects of H₂, which have been reviewed elsewhere (LeBaron et al., 2024). As stated above, H₂ is often administered as HRW. When HRW is ingested, the H₂ molecules diffuse through the intestinal submucosa, quickly enter the bloodstream, and appear in exhaled breath within a few minutes (Mikami et al., 2019). This early work on H₂ is worth considering for the discussion on agricultural systems. As will be discussed below, the effects of H₂ on cellular activities will be similar in plants and animals. Much of the H₂ research is on animals and biomedical uses, and much can be learned from this to translate into the plant science arenas.

The consumption of HRW has been widely reported to provide many health benefits including cardiovascular metabolic diseases (Singh et al., 2024). Moreover, the bio-accessibility and bio-availability of nutrients are fundamental properties of foods utilized in metabolic processes. Recent studies propose that drinking hydrogen-rich water after a meal, including fruits, vegetables, meats, and other foods, may enhance the bio-accessibility and bio-availability of various nutrients and vitamins (Alwazeer, 2024a).

2.2 H₂ and agriculture

After the promising results of hydrogen's impact on health, researchers have become increasingly interested in its potential applications in agriculture. Early studies in China have explored the potential uses of H₂ in agriculture and its impact on crop production.

2.2.1 Effects of H₂ on soil bacteria

Hydrogen is used as an energy source by diverse bacteria in soils for growth and survival (Greening et al., 2022). Soil bacteria drive hydrogen cycling in agricultural systems (Islam Z. F. et al., 2023). Such bacteria are an important sink for atmospheric H₂, accounting for ~75% of H₂ uptake. Paradoxically, soil bacteria are also the main contributors to H₂ emissions from soil due to the production of H₂

by fermentation and nitrogen fixation. Elevated H₂ exposure indeed altered soil microbial community structure and diversity (Wang et al., 2020; Xu et al., 2021). As expected, H₂ promoted the enrichment of H₂-oxidizing bacteria (HOB), some of these species act as plant-growth promoters demonstrated to increase root elongation in wheat seedlings and plant biomass in *Arabidopsis* (Maimaiti et al., 2007). These HOB isolates exert 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, phosphorus solubilization, siderophores enhancement, and antifungal activity, leading to plant nutrient absorption and growth (Maimaiti et al., 2007; Fan et al., 2022; Wu et al., 2022).

In addition, Stein et al. (2005) showed an increase in net CO₂ fixation in soils was achieved with the addition of H₂. Specific marker genes associated with microbial carbon fixation, denitrification, and nitrogen fixation were further observed to be more abundant in H₂-treated soils (Xu et al., 2021). In contrast, Wang et al. (2020) reported that the variation in abundance of selected nitrogen-cycling genes was attributed, in part, to significant differences in soil moisture rather than H₂ infusion under field conditions. Moreover, soil enzyme activities (Liu et al., 2010) and organic pollutant degradation (e.g., polychlorinated biphenyls) (Xu et al., 2020) were enhanced in response to H₂ infusion. Therefore, H₂ may influence soil microbial community structure and functions, and may indirectly improve soil health, promoting both plant growth and yield.

2.2.2 Effects of H₂ on plant growth and yields

An increasing body of studies show that H₂ may play a vital role in plant growth, developmental processes, and stress responses. This indicates that H₂ may be an effective alternative or adjunctive treatment to current pesticide and fertilizer inputs, helping to promote nutritional content and yield to a certain extent. In plants, H₂ can evolve under normal or various stress conditions resulting from phytohormone induction (Xie et al., 2012; Zeng et al., 2013; Jin et al., 2016). H₂ was involved in the signaling pathways of phytohormones (such as auxin, abscisic acid, gibberellin, and salicylic acid (SA)), ROS, NO \bullet , carbon monoxide (CO), hydrogen sulfide (H₂S), and melatonin, resulting in improvement of plant rooting and tolerance against various biotic and abiotic stresses that limit crop yield (Xie et al., 2014; Jin et al., 2016; Wu et al., 2020a; Zhang et al., 2020; Su et al., 2021; Shao et al., 2023). For instance, rice stripe virus (RSV) is rice's most devastating plant virus, leading to severe losses in field production. External H₂ supply was observed to confer rice resistance to RSV through an SA-dependent pathway (Shao et al., 2023).

Evidence of the above H₂ effects were supported by using transgenic *Arabidopsis* plants overexpressing a hydrogenase gene from *Chlamydomonas reinhardtii* (Zhang et al., 2020; Su et al., 2021; Shao et al., 2023). These results indicated that H₂ might be a component of the survival strategy of plants through enhancing responses to biotic and abiotic stress. Field trials further confirmed that HRW irrigation could improve the size and weight of rice grains by regulating the transcriptional profiles of representative genes controlling quantitative traits. These include heterotrimeric G protein β -subunit (RGB1), grain size 3/5 (GS3/5), small grain 1 (SMG1), grain weight 8 (GW8), and nitrogen, phosphorus, and potassium assimilation or transport-related genes (Cheng et al., 2021). Recent research has also found that nitrate reductase (NR)

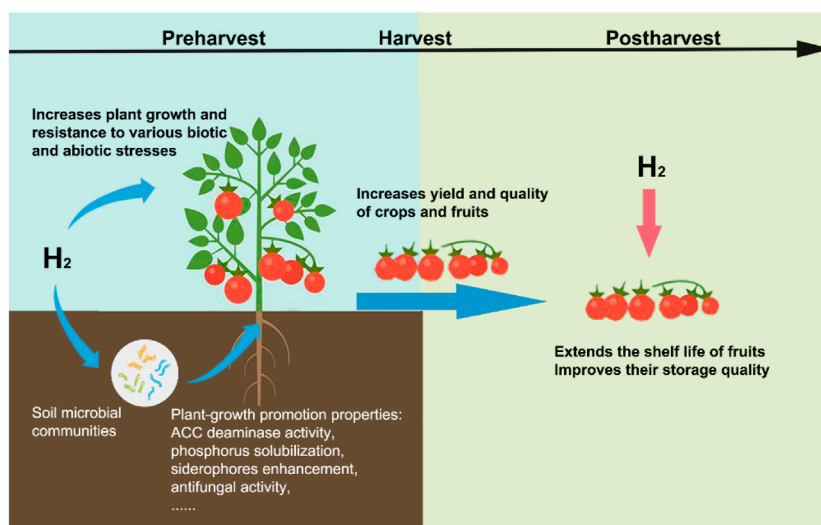


FIGURE 2 Application of H₂ in the agri-food chain. ACC: 1-aminocyclopropane-1-carboxylate.

might be a target of H₂ sensing, positively regulating nitrate uptake and seed size in *Arabidopsis thaliana* (Cheng et al., 2023). Li et al. (2021b) identified that the fertilization effect of H₂ resulted in the proliferation of plant-growth-promoting rhizobacteria, and the enhancement of plant nutrient uptake and use efficiency, indicating that H₂ has the potential to be an alternative fertilizer to some extent and, in turn, reducing the use of chemical fertilizers (Li et al., 2021b) (Figure 2).

As can also be seen in Table 1, a range of biological effects is seen in plants when H₂ is used as a treatment. The noted effects range from the modulation of seed germination (Xu et al., 2013; Huang et al., 2021), seed size (Cheng et al., 2023), stress tolerance (Zeng et al., 2013), flower senescence (Su et al., 2019), and even postharvest storage of plant materials (Hancock et al., 2022). Therefore, adding H₂ to various stages of food production, from adding it to feedwater to gassing food products, may be advantageous.

Whatever the mechanism(s), they need to account for how H₂ can lead to many effects in plants, including increased growth and stress tolerance, delayed senescence, and altered gene expression (Figure 3).

2.2.3 Effects of H₂ on heavy metal and nitrogen compound accumulation and toxicity in plants

Excessive use of chemical fertilizers and pesticides has seriously threatened environmental health and food safety (Baweja et al., 2020). As a result, heavy metals accumulate in crop plants, severely threatening human health through food chains. It has been showed that cadmium (Cd) accumulation can be inhibited by H₂ in seedlings of alfalfa (Cui et al., 2013), Chinese cabbage (Wu et al., 2015), rapeseed (Zhao G. et al., 2021), and cucumber (Wang et al., 2019). H₂-modulated sulfur compound metabolism, redox homeostasis, and Cd transportation were mainly responsible for alleviating Cd toxicity (Cui et al., 2013; Cui et al., 2020; Wu et al., 2021). Furthermore, HRW irrigation was observed to decrease Cd accumulation in rice grains through down-regulating Cd

transport-related genes in field trials (Cheng et al., 2021). Besides this, nitrogen fertilizer overuse leads to nitrate and nitrite accumulation. H₂ can regulate the expression of nitrate transporter genes (*BcNRT1.5* and *BcNRT1.8*), simultaneously increasing activities of nitrate reductase (NR) and glutamine synthetase, resulting in a decrease in nitrate transport from roots to shoots and, in turn, the decreased accumulation of Cd in Chinese cabbage seedlings (Wei et al., 2021). During the storage of tomato fruits, nitrite accumulation was also prevented by H₂, which was attributed to suppressed (NR) or increased (nitrite reductase (NiR)) activities and their corresponding coding genes (Zhang et al., 2019). In addition to the accumulation of Cd through excessive use of fertilizers, pesticide residues are another major threat to food safety. It has been found that H₂ can enhance the degradation of the pesticides chlorothalonil and carbendazim in the leaves of tomato seedlings, but importantly this does not reduce their antifungal efficacy (Wang et al., 2022; Zhang et al., 2022). Further pharmacological and genetic evidence has confirmed the contribution of H₂-stimulated brassinosteroids (Wang et al., 2022) and glutathione (Zhang et al., 2022) in the above responses.

2.2.4 Effects of H₂ on secondary metabolism

With improving living standards, consumers are paying closer attention to the quality of agricultural products. While the use of fertilizers and pesticides boosts crop productivity, these methods can simultaneously diminish the quality of the crops. Phenylpropanoid metabolism is one of the most important secondary metabolic pathways in plants. The products of these processes include anthocyanin, flavonoids, and phenolics known to contribute to plant development and environment interplay, as well as food quality and health-promoting actions. Studies have shown that H₂ increased anthocyanin and flavonoid accumulation in radish sprout hypocotyls and alfalfa seedling leaves under UV-A and UV-B, respectively, by regulating the expression of genes associated with phenylpropanoid metabolic pathway (Su et al., 2014; Xie et al.,

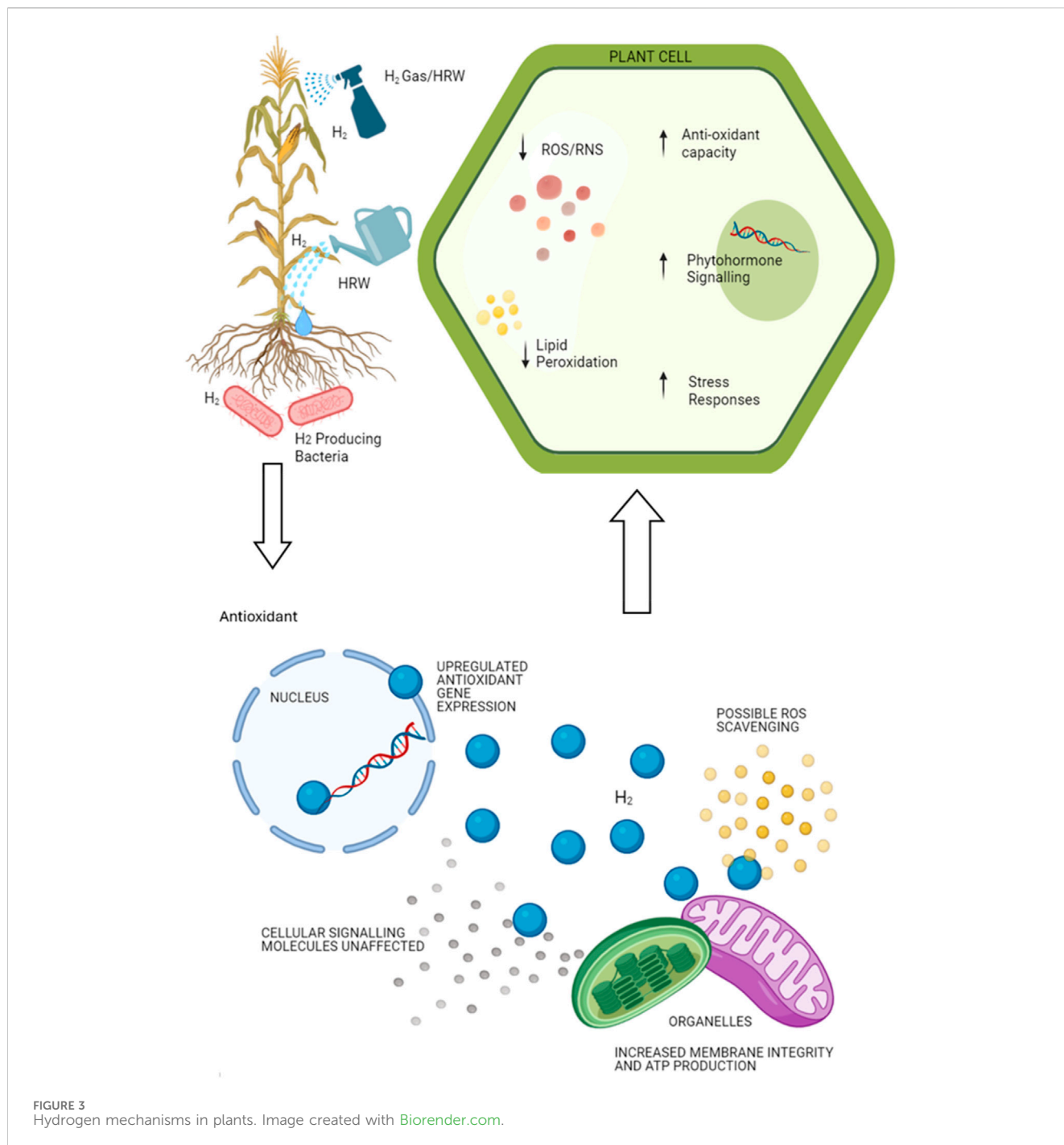
TABLE 1 The impact of hydrogen application on plant growth and development.

Crop	Treatment	Environment	Effect	Potential	References
Brassica					
<i>Arabidopsis thaliana</i>	H ₂ -oxidizing bacteria inoculation of soil	Laboratory: Soil (No challenge)	- Increased biomass and leaf development	- Similar soil benefits as legume-crop rotational planting	Maimaiti et al. (2007)
<i>Arabidopsis thaliana</i>	HRW (0.59 mM)	Greenhouse: HRW irrigation (RSV challenge)	- Increased salicylic acid content	- To promote resistance to viral infection	Shao et al. (2023)
Chinese cabbage	HRW (0.4 mM)	Laboratory: Nutrient solution (Cd challenge)	- Increased antioxidant effect	- To promote germination and plant establishment - Reduce the effects of Cd toxicity	Wu et al. (2015)
Chinese Cabbage	HRW (0.4 mM)	Laboratory: Soil (Ca(NO ₃) ₂ challenge)	- Increased antioxidant effect	- To promote plant establishment - Reduce the effects of fertilizer nitrite toxicity	Wei et al. (2021)
Cereal					
Barley	HRW (0.8 mM)	Laboratory: Nutrient solution (NaCl challenge)	- Increased Na ⁺ extrusion - Increased K ⁺ retention	- To promote plant establishment - To increase resistance to salinity challenge	
Barley	HRW (2.0 mM)	Laboratory: Seed priming HRW (No challenge)	- Increased bioactive compounds (e.g., free vanillic acid, coumaric acid)	- To increase germination efficiency at low temperatures	Guan et al. (2019)
Rice	HNW (0.5 mM)	Field: Soil irrigation (Cd challenge)	- Increased quantity of rice grain (field and grain) - Maintained nutritional elements - Alleviated Cd accumulation	- To enhance crop production - To alleviate heavy metal toxicity	Ma et al. (2021)
Rice	HRW (0.59 mM)	Greenhouse: Nutrient solution (RSV challenge)	- Increased salicylic acid content	- To promote resistance to viral infection	Shao et al. (2023)
Rice	HRW (unknown)	Greenhouse: HRW irrigation	- Increased vegetative fitness parameters	- To regulate plant growth and increase stress-resistance	Liu et al. (2017)
Wheat	HRW (0.75 mM)	Laboratory: Seed priming HRW (Drought challenge)	- Increased chlorophyll, iron, zinc and soluble protein concentrations	- To promote plant growth in arid climates	Islam et al. (2023a)
Wheat	H ₂ -oxidizing bacteria inoculation of soil	Laboratory: Soil (No challenge)	- Improved root elongation	- To promote germination and plant establishment	Maimaiti et al. (2007)
Fruit					
Cucumber	HRW (0.21 mM)	Laboratory: Petri-dish (No challenge)	- Upregulated nitrite reductase expression	- To promote germination and plant establishment	Zhu et al. (2016)
Cucumber	HRW (0.25–4 μM)	Laboratory: Petri-dish (Cd challenge)	- Increased antioxidant effect - Increased osmotic adjustment substances content	- To promote adventitious rooting and plant establishment - Reduce the effects of Cd toxicity	Wang et al. (2019)
Tomato	HRW (0.4 mM)	Greenhouse: Soil irrigation (No challenge)	- Increased sucrose, starch, soluble sugar, soluble protein content - Decreased cellulose, hemicellulose, lignin content	- To increase nutrient content of produce	Yao et al. (2024)
Tomato	HRW (0.8 mM)	Greenhouse: Soil irrigation (Fungicide challenge)	- Enhanced degradation of pesticides	- To improve resistance to fungicide toxicity - Improve pathogen resistance	Wang et al. (2019)

2015). Metabolomic and transcriptomic profiles revealed that transcription factor genes related to phenylpropanoid biosynthesis and metabolism (including *MYBs*, *bHLHs*, and *WRKYs*) were also involved in H₂-increased coumarins and

flavonoids accumulation in Wuzhimaotao (*Ficus hirta* Vahl; a common Chinese herbal medicine) (Zeng and Yu, 2022).

Further field trials confirmed that HRW irrigation enhanced strawberries' specific volatile compound levels, sugar-acid ratio, and



sensory attributes, with/without fertilizer application (Li L. et al., 2022). Notably, the adverse effects of fertilizers on strawberry fruit aroma were alleviated after HRW irrigation.

2.2.5 Effects of H₂ on nutrient levels in plants

HRW irrigation also increased the content of some nutrient elements, including phosphorus, potassium, magnesium, and iron, in white rice (Cheng et al., 2021). After 1 year of storage, the HRW-irrigated rice had higher levels of essential amino acids, especially lysine, and lower off-flavors (Cai et al., 2022). Therefore, H₂ supplementation may represent a novel and low-carbon strategy

applicable to enhancing the production and preservation of crops, vegetables, and fruits in the agri-food chain.

2.2.6 Further thoughts on H₂ effects on plants

It is worth noting that H₂ effects may vary depending on the species, genetic background, and original habitats of plants (Li L. et al., 2022). Although many unknowns exist regarding the effects of extra H₂ input on plants and soils and how agricultural practices affect them, there is currently no evidence that excess H₂ causes adverse effects on plant physiology and soil microbial community composition. It will be crucial to consider the optimal H₂ dosage in

future agricultural applications, mitigating any potential contribution to an increased proliferation of H₂-oxidising plant pathogens or the inhibition of plant growth and development. Furthermore, considering the optimal dosing with H₂ will also help to minimize the cost/benefit of its use and, therefore, encourage its uptake in the agricultural industries.

2.3 H₂ and food

The idea that H₂ can exert biological effects coincides with an evolutionary perspective. For example, the role of hydrogen gas in microbiology has played a key role in both the genesis of life billions of years ago as well as in the evolution of prokaryotes and eukaryotes (Martin and Müller, 1998). Some microorganisms (e.g., algae, archaea, bacteria, protozoa) and most plants contain hydrogenase enzymes that can generate or consume molecular hydrogen. A mechanism which is suggested to play a role in maintaining redox homeostasis (LeBaron et al., 2023). This evolutionary insight opens up intriguing possibilities for modern applications, from enhancing plant growth in agriculture to improving food packaging technologies. Understanding the historical and evolutionary context of hydrogen's biological effects enriches our appreciation of its potential and guides future research and application strategies.

Although the use of H₂ in biology, foods, and agriculture is recent, the biological effects of hydrogen were reported in the late 18th century (Hancock and LeBaron, 2023; LeBaron et al., 2023). Hydrogen-enriched water, in the form of electrolyzed water, was first documented in an agriculture as early as 1931 (Hancock and LeBaron, 2023). This historical context underscores a longstanding interest in harnessing hydrogen for various biological applications.

In food technology, H₂ was first introduced in the hydrogenation process of vegetable oils to produce vegetable shortening and margarine (Coenen, 1976). Apart from this use, no other application of hydrogen could be found in the food industry field. This may be because H₂ is chemically inert under standard conditions, and safety concerns are related to forming explosive mixtures with air.

Hydrogen studies in microbiology started in the second half of the 20th century. The effect of H₂ on microbial growth and heat resistance first appeared in scientific literature in the 1980s.

In 1983, in the United States, two researchers from the Department of Food Science and Nutrition, University of Minnesota, studied the effect of different gases, including H₂, on the germination of *Clostridium botulinum* spores and found H₂ reduced the oxidoreduction potential in the experimental system (Foegeding and Busta, 1983). In 1990, another United States team from the University of Wisconsin revealed that H₂ could accelerate the thermal inactivation of *Clostridium botulinum* II3B spores (Kihm and Johnson, 1990). Further to this, in 1998, a research team from the Institute of Food Research, United Kingdom, used H₂ in a study regarding the effect of oxidoreduction potential (redox) on the recovery of sub-lethally heat-damaged cells of different pathogens, including *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* (George et al., 1998), identifying that the heat resistance of all studied bacteria was higher in an H₂-included atmosphere than in an O₂-included one.

Early studies on the potential use of H₂ in the food industry appeared in the 1990s in France when researchers in the Microbiology Laboratory of ENSBANA, Université de Bourgogne, supervised by the French microbiologist Charles Diviès, were examining the effect of redox potential on the metabolism processes of Lactic Acid Bacteria (LAB). As the LAB is used for the production of different dairy products, Diviès's team planned to evaluate the impact of redox potential on the microbial quality, aroma, and shelf life of these products. As the region where the Diviès laboratory is located, i.e., Bourgogne, is famous for its wine, the team researched redox's impact on the growth and the metabolism of *Saccharomyces cerevisiae* (Capelle, 2001). Shortly after, the research topics were expanded to include new food product groups such as ready-to-eat salads (Ouvry, 2001; Ouvry et al., 2002), fruit juice (Alwazeer, 2003; Alwazeer et al., 2003), pickles (Olsen, 2008), wine (Pham et al., 2008), dairy beverage (Giroux et al., 2008), milk gel (Martin et al., 2009), yogurt (Martin et al., 2010; 2011), and fermented milk (Ebel et al., 2011). In these different research topics of redox, hydrogen gas was used as a redox modifier (reducing agent) of the medium/product.

The aim of Diviès's team was not to study the impact of hydrogen but instead change the redox potential of the medium by sometimes using gases, such as H₂ and N₂, and other times chemicals such as dithiothreitol, sodium borohydride, and ferricyanide. Hydrogen was used as a reducing agent to lower the redox value of the medium while oxidizing agents such as O₂ and ferricyanide were used to raise it.

Since 2017, Alwazeer, a member of Diviès's team, has created a new hydrogen research group in Turkey and has been exploring new food products/processes. The driving force of the new team was the promotion of the results of hydrogen's effect in health and agriculture fields. In 2017, Alwazeer's hydrogen team started to explore the potential applications of H₂ in different food technologies, including drying, packaging, safety, and phytochemical extraction (Figure 4).

3 Hydrogen as a sustainable strategy for agriculture

There is no doubt that plants can respond to H₂; indeed, it has been suggested to be suitable and advantageous for wide use in agriculture (Li et al., 2021a).

3.1 Methods of H₂ application in agriculture

Exposure and treatment of plants with H₂ can be carried out in a variety of methods, from H₂-producing bacteria (Maimaiti et al., 2007), using it as a gas, having water or aqueous feed enriched with H₂ (hydrogen-rich water (HRW)), or creation of nanobubbles (hydrogen nanobubble water (HNW)), as outlined in Table 2. Each of these has its benefits but also issues. For example, H₂ gas is very light and will rapidly rise in the atmosphere, limiting the exposure time at ground level. This presents challenges for fumigation, particularly over large areas, as H₂ would dissipate too quickly in an open field. In enclosed environments, such as

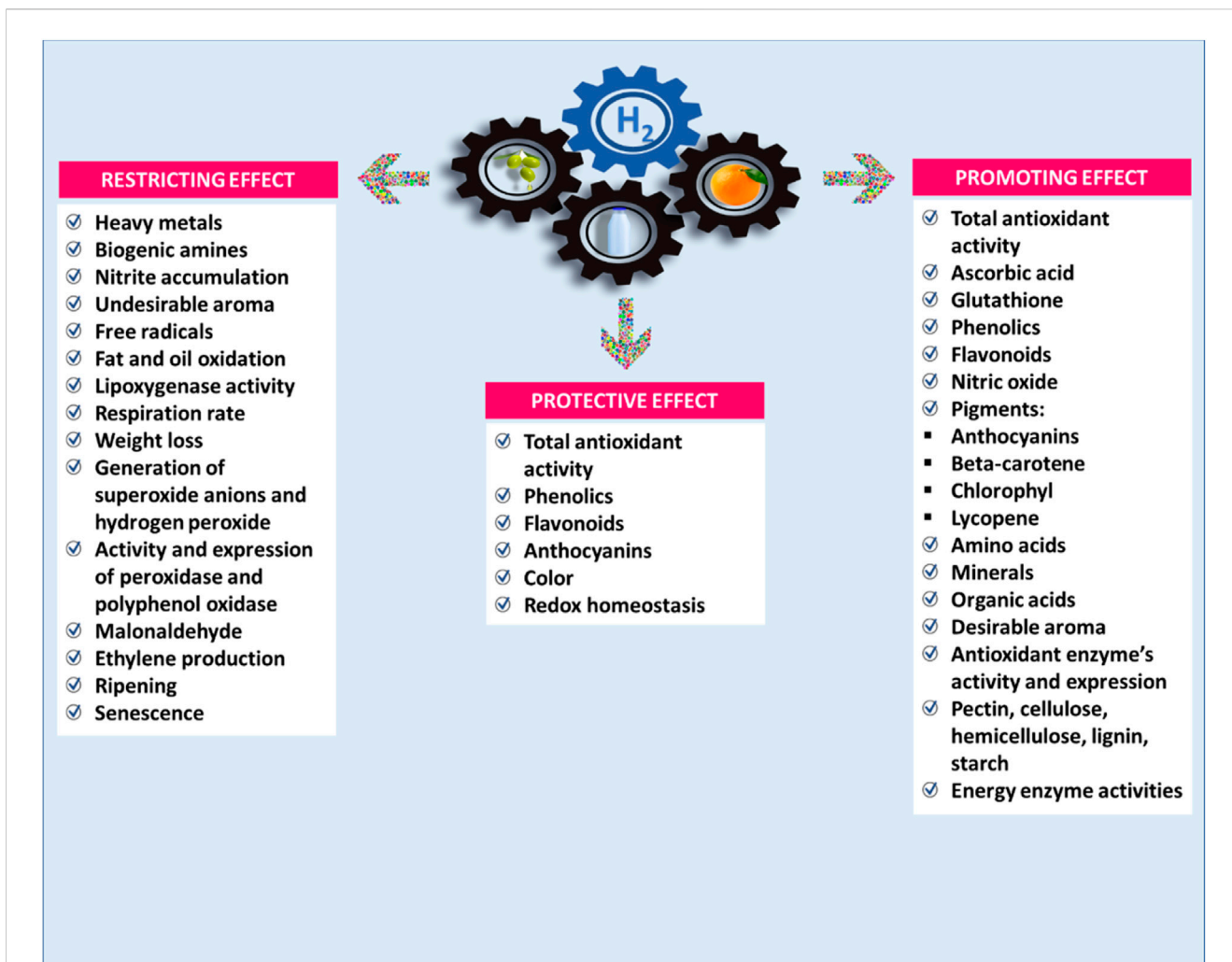


FIGURE 4 Effects of molecular hydrogen on food products.

TABLE 2 Examples of common methods for treating plants with H₂.

Method of treatment	Effect in plants seen	Reference(s)
As a gas	Stimulated plant growth, increased biomass, and improved root development (<i>Arabidopsis thaliana</i>)	Maimaiti et al. (2007)
Hydrogen-rich water (HRW)	Improved seed germination in cucumber (<i>Cucumis sativus</i> L.)	Huang et al. (2021)
	Improved seed germination under salt stress in rice (<i>Oryza sativa</i> L., Wuyunjing 7)	Xu et al. (2013)
Hydrogen-rich saline (HRS)	It can be used for salinity stress experiments in plants	Zheng et al. (2009)
Nanobubble water	Delays petal senescence and prolongs vase life of flowers (<i>Dianthus caryophyllus</i> L.)	Li et al. (2021a)
Genetic manipulation	<i>Arabidopsis CrHYD1</i> lines (Gene ID: 5,718,949; hydrogenase gene from <i>Chlamydomonas reinhardtii</i>)	Cheng et al. (2023)

greenhouses, the flammability of H₂ in air poses significant safety concerns. However, as H₂ becomes more prevalent as a fuel, future protocols are expected to mitigate these flammability issues.

3.1.1 Treatment with hydrogen-rich water (HRW)

Cells need to be exposed to H₂ to have action, which poses challenges for H₂ use in agriculture. It is relatively easy to deliver H₂ to plants in the form of HRW (or HNW), as it can be sprayed onto

foliage or straight on the soil as feed water. However, H₂ may not stay in solution very long (Russell et al., 2024), and therefore, using HRW (or HNW) may have a short timeframe for facilitating H₂ exposure.

HRW is relatively unstable, and H₂ will rapidly enter the gas phase and limit the shelf-life of the HRW solution and, therefore, its effectiveness, as mentioned above. A variation of this is to use hydrogen-rich saline (HRS), which has been used in animal research

TABLE 3 Examples of the effect of hydrogen application in postharvest crops and food products.

Crop/food product	H ₂ application method (°conc.)	Main outcomes	Country	Reference(s)
Polyunsaturated fatty acids enriched dairy beverage	H ₂ bubbling 4% v/v mixed gas; (°31 µM)	- Reduction of oxidative degradation - Color protection	Canada	Giroux et al. (2008)
Kiwifruit	HRW; 80% (°624 µM)	- Alleviation of pectin solubilization and reduction of the activities of cell wall-degrading enzymes - Reduction of the respiration intensity - Increase of the activity of superoxide dismutase - Decrease of lipid peroxidation level - Maintenance of the radical scavenging activity - Higher integrity of the inner membrane of mitochondria	China	Hu et al. (2014)
Kiwifruit	H ₂ fumigation	- Delay of the softening and cell wall disassembly in flesh - Inhibition of ethylene production - Decrease in 1-aminocyclopropane-1-carboxylate (ACC) concentration, ACC synthase, and ACC oxidase activities, and downregulation of the corresponding gene transcripts - Decrease in the natural decay and disease incidence	China	Hu et al. (2018) ; Yun et al. (2022) ; Dong et al. (2023b)
Fresh-cut kiwifruit	HRW; 100% (°780 µM)	- Delay of the decrease in titratable acidity, chlorophyll, ascorbic acid, total phenolic, and total flavonoid contents - Reduction of the total colony numbers and weight loss - Inhibition of the soluble solid and malondialdehyde content increases, and electrolyte leakage increases - Maintenance of the green color and firmness	China	Zhao et al. (2021b)
<i>Rosa sterilis</i> fruit	HRW; 60% (°468 µM)	- Retarding the increase in decay incidence, weight loss, respiration rate, and malondialdehyde content - Slowdown of the decrease of titratable acid, total soluble solids, and firmness - Reduction of hydrogen peroxide and the superoxide anion production - Increasing the activity and expression of antioxidant-related enzymes (superoxide dismutase, catalase, dehydroascorbate reductase, glutathione reductase, ascorbate peroxidase, and reductase) - Increase in the ascorbic acid and glutathione levels - Reduction of the content of oxidized glutathione and dehydroascorbate - Increase in the activities and expression of H ⁺ -ATPase, succinate dehydrogenase, Ca ²⁺ -ATPase and cytochrome C oxidase - Promotion of levels of ADP and ATP. - Decrease in the AMP content	China	Dong et al. (2022)
Fresh-cut Chinese water chestnut	HRW; 100% (°780 µM)	- Delay of the yellowing - Reduction of the H ₂ O ₂ accumulation and lipoxygenase activity - Enhancement of the superoxide dismutase activity - Slowdown of the accumulation of flavonoids and transcript levels of phenylpropanoid pathway genes	China	Li et al. (2022a)
Banana	HRW; 400 µM	- Lower expressions of cell wall degradation-related genes - Higher levels of fruit firmness, pectin,	China	Yun et al. (2022)

(Continued on following page)

TABLE 3 (Continued) Examples of the effect of hydrogen application in postharvest crops and food products.

Crop/food product	H ₂ application method ^(a) (conc.)	Main outcomes	Country	Reference(s)
		<ul style="list-style-type: none"> hemicellulose, and lignin - Higher levels of starch - Lower level of total soluble solids (TSS) - Lower expression of flavor-related genes - Delay of the degradations of starch and cell walls 		
Edible mushroom (<i>Hypsizygus marmoreus</i>)	HRW; 25% (*195 μM)	<ul style="list-style-type: none"> - Reduction of the relative electrolyte leakage rate and malonaldehyde content - Increase in the activities and gene expression of antioxidants, superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase 	China	Chen et al. (2017); Yun et al. (2021)
Tomato	HRW; 50% and 75% (* 390 μM and 585 μM)	<ul style="list-style-type: none"> - Reduction of the disease injury of <i>Botrytis cinerea</i>-inoculated tomato fruit and the lesion areas - Increase in the polyphenol oxidase activity - Increase in the nitric oxide content - Enhancement of the defense responses of tomato fruit to <i>Botrytis cinerea</i> 	China	Lu et al. (2017)
Tomato	HRW; 585 μM	<ul style="list-style-type: none"> - Delay of the decreased fruit H₂ production and senescence - Blocking of nitrite accumulation and the decrease in vitamin C - Inhibition or increase of activities and transcripts of nitrate reductase and nitrite reductase 	China	Zhang et al. (2019)
Black barley	HRW; 1,000 μM	<ul style="list-style-type: none"> - Increasing the germination rate and growth rate - Increasing the concentrations of free vanillic acid, coumaric acid, sinapic acid, conjugated sinapic acid, Ca and Fe, and the hydroxyl radical scavenging rate - Decreasing protein, fat, starch, and dietary fiber contents 	China	Guan et al. (2019)
Okra	HRW; 100% (*780 μM)	<ul style="list-style-type: none"> - Maintenance of fruit firmness - Delay of softness - Extension of shelf life - Upregulation of genes involved in the biosynthesis of pectin, hemicellulose and cellulose - Downregulation of numerous cell wall degradative genes 	China	Dong et al. (2023c)
Okra	HRW; 100% (*780 μM)	<ul style="list-style-type: none"> - Delay of senescence - Maintenance of fruit quality during storage - Upregulation of all of the melatonin biosynthetic genes - Lower abscisic acid content 	China	Dong et al. (2023b)
Orange juice	H ₂ bubbling 4% v/v mixed gas; (*31 μM)	<ul style="list-style-type: none"> - Protection of ascorbic acid and color quality 	France	Alwazeer (2003); Alwazeer et al. (2003)
Non-fat yogurt	H ₂ bubbling 4% v/v mixed gas; (*31 μM)	<ul style="list-style-type: none"> - Modification of aroma profile 	France	Martin et al. (2011)
Acid skim milk gel	H ₂ bubbling 4% v/v mixed gas; (*31 μM)	<ul style="list-style-type: none"> - Decrease of whey separation and aggregation of proteins within the matrix - Decrease in whey separation 	France	Martin et al. (2009)
Yogurt	H ₂ bubbling 4% v/v mixed gas; (*31 μM)	<ul style="list-style-type: none"> - Increase in <i>B. bifidum</i> survival during storage without affecting the fermentation kinetics and the survival of <i>Strep. Thermophilus</i> and <i>L. delbrueckii</i> ssp. <i>Bulgaricus</i> 	France	Ebel et al. (2011)

(Continued on following page)

TABLE 3 (Continued) Examples of the effect of hydrogen application in postharvest crops and food products.

Crop/food product	H ₂ application method (°conc.)	Main outcomes	Country	Reference(s)
Minced meat	H ₂ -producing magnesium	- Control of the microbial growth of minced beef meat - Restriction of the formation of thiobarbituric acid reactive substances - Restriction of the formation of biogenic amines - Restriction of the formation of the off-volatile compounds - Protection of the whole quality of MBM	Turkey	Çelebi et al. (2024)
Cultured butter	HRW; 100%, and Mg water washing (°780 µM)	- Protection of physicochemical properties, oxygen-sensitive bioactive compounds, unsaturated fatty acids, total quality properties, and antioxidant content	Turkey	Ceylan et al. (2022)
Cultured butter	HRW; 100%, and Mg water washing (°780 µM)	- Reduction of the heavy metal content	Turkey	Alwazeer et al. (2022b)
Cultured butter	HRW; 100%, and Mg water washing (°780 µM)	- Restriction of the biogenic amine formation	Turkey	Bulut et al. (2022)
Cultured butter	H ₂ bubbling 100% v/v (°780 µM)	- Preservation of the color (yellowness) during cold storage - Increase of the chemical and microbial stability during cold storage - Restriction of the formation of biogenic amines by 44%–61% - Prevention of the undesirable volatile compound formation - Extension of the shelf life by about 30 days	Turkey	Alwazeer et al. (2024)
Fermented beetroot	HRW; 100% (°780 µM)	- Restriction of the biogenic amine formation	Turkey	Alwazeer et al. (2022a)
Rice milk	HRW; 100% (°780 µM)	- Change of mineral, amino acid, volatile, and sugar profile	Turkey	Zor et al. (2023)
Fresh cheese	H ₂ (4% vol) atmosphere packaging (°31 µM)	- Preservation of the freshness of fresh cheese	Turkey	Alwazeer et al. (2020)
Strawberry	H ₂ (4% vol) atmosphere packaging (°31 µM)	- Extension of shelf life - Preservation of the nutritional, textural, and freshness properties	Turkey	Alwazeer and Özkan (2022)
Fish (Rainbow trout and Horse mackerel)	H ₂ (4% vol) atmosphere packaging (°31 µM)	- Restriction of the biogenic amine formation	Turkey	Sezer et al. (2022)
Apricot	H ₂ (4% vol) atmosphere drying (°31 µM)	- Preservation of the color, nutritional content, antioxidant capacity, and sensory properties of dried product	Turkey	Alwazeer (2018), Alwazeer and Örs (2019)
Beer	H ₂ (4% vol) gassing (°31 µM)	- Foam retention property	United States	Girault et al. (2013)
H ₂ -infused beverage	H ₂ ; bubbling 100% v/v (°780 µM)	- Improvement of performance, delay of muscle fatigue - Improvement of cognitive function of consumer (focus, speed, plasticity)	United States	LeBaron et al. (2021)

°Concentration calculated based on the reported percent of an assumed (not reported) saturation of hydrogen gas in pure water under standard ambient conditions, which is approximately 780 µM (LeBaron et al., 2024).

(Zheng et al., 2009) but may be helpful for salt stress experiments in plants (e.g., Wu et al., 2020b). However, the longevity of HRS is still an issue. Nanobubble technology will partly mitigate the stability of hydrogen-rich solutions, but it is still not stable in the long term (Russell et al., 2024).

3.1.2 Hydrogen-producing nanoparticles

H₂ can be supplied using donor molecules, such as ammonia borane-loaded hollow mesoporous silica nanoparticles (AB@

hMSN), as used by (Wang et al., 2021) on tomatoes. Alternatively, magnesium hydride (MgH₂) can be used, as reported, for the treatment of cut flowers (Nguyen and Lim, 2022). However, the use of such materials brings challenges, too. However, if donor molecules are used, by-products will be left behind, either in the plant or the environment, and such by-products may be inherently toxic. Therefore, spraying large quantities of such donor molecules onto field crops may not be desirable. It is worth noting here, too, that the use of donor

molecules is most likely to cost more, and therefore, the overall cost/benefit analysis may be less financially favorable, if favorable at all.

Therefore, at least in the near future, the most likely method for H₂ application in agriculture is going to be HRW or HNW. HNW can be produced quite simply. It has been reported that H₂ delivered to solution through a bamboo cane (Shin et al., 2016) can create nanobubbles, which increases the persistence time of H₂ in solution, making it more desirable as a delivery system. However, in the future, safe and easy-to-use H₂ donors may be developed, which will increase the effectiveness of H₂ treatments, ease their delivery, and potentially provide a better cost/benefit outcome, although sustainability issues with donor molecules will also need to be considered. For any method adopted in agriculture, the cost/benefit analysis must make its use attractive. However, as the manufacturing, usage, and storage of H₂ becomes more common and less expensive, its use in biological systems, such as in agriculture and medicine, may increase. For example, in addition to its potential use both pre- and postharvest, H₂ is being explored as a vehicle fuel source (Singla et al., 2021), which may reduce the emissions from, and the costs associated with, fuel consumption when transporting perishable goods. It is clear that H₂ has many positive effects on plants and food products, and its use is safe for human health as long as the safety of H₂ is considered.

3.2 H₂ and the preservation of postharvest crops

As has been discussed above, H₂ may be used as a gas-enriched water solution, referred to as hydrogen-rich water (HRW), and has been utilized widely (Table 3). One of the most potentially exciting uses of H₂ is in the storage of postharvest plant materials, such as fruit and vegetables.

Hu et al. (2014) studied the effects of HRW on prolonging the shelf life of kiwifruit and the underlying mechanisms. Their study showed that pre-treatment with 80% HRW (0.6 mM) could effectively reduce the rot incidence and inhibit postharvest kiwifruits' respiration intensity. Specifically, the 80% HRW treatment delayed the decrease in kiwifruit firmness by alleviating pectin solubilization and suppressing cellulase, polygalacturonase, and pectin methylesterase enzyme activities. Additionally, the HRW treatment reduced lipid peroxidation by increasing the SOD activity and free radical scavenging activity. Thus, the results demonstrate that HRW treatment could delay fruit ripening and senescence during storage by regulating the antioxidant defense. In a comprehensive study conducted by (Dong et al., 2022), the application of HRW (20%, 60%, 100%; 0.12 mM, 0.36 mM, and 0.6 mM, respectively) was found to influence the preservation of *R. sterilis* fruit quality. HRW treatment significantly delayed the decline of total acidity (TA), total soluble solids (TSS), and fruit firmness. Additionally, H₂ application effectively postponed the onset of decay, weight loss, increased respiration rate, and malondialdehyde (MDA) accumulation. Moreover, there was a concurrent increase in the activities and expression of enzymes associated with antioxidant systems. Further analysis also indicated elevated adenosine diphosphate (ADP) and adenosine triphosphate (ATP) levels in treated fruit. These findings suggest that HRW treatment regulates the antioxidant capacity and energy status

during storage at ambient temperature and holds significant promise as a preservation strategy for *R. sterilis* fruit. Dong B. et al. (2023) conducted further studies exploring the impact of varying concentrations of HRW at 0, 0.2, 0.4, and 0.8 mM on the quality maintenance of fresh-cut *Gastrodia elata* during storage at a low temperature (4°C). The findings revealed that the application of HRW effectively inhibited the decline in weight loss and the increase in respiration rate. HRW also slowed down the reduction in total acidity and soluble solids over the storage period. Furthermore, the HRW treatment minimized the production of O₂[•] and H₂O₂, increasing the activity and expression of various antioxidant-related enzymes. HRW also increased the levels of non-enzymatic antioxidant substances such as ascorbic acid and glutathione (GSH). Concomitant reduction in the activity and expression of peroxidase and polyphenol oxidase were also observed. Moreover, HRW treatment hindered the decline in ADP and ATP content and energy status, and slowed down the reduction in H⁺-ATPase, succinate dehydrogenase, Ca²⁺-ATPase, and cytochrome oxidase activities, substantiating that HRW treatment can preserve the postharvest quality of fresh-cut *G. elata*.

4 Hydrogen in food processing and packaging

The growing awareness of consumers towards the health risks of synthetic food additives has encouraged food processors to look for effective "green" or "natural" innovative alternatives. H₂ possesses many specific physical, chemical, and biological properties. The small size, high diffusibility, and chemical neutrality, besides the biological activities of H₂, have opened the door for many potential applications in the food industry. In recent years, several studies have been conducted to explore the effect of H₂ on the quality, safety, and shelf-life of food products.

4.1 H₂ and food drying

In food drying, the inclusion of H₂ in the drying atmosphere, besides CO₂ and N₂ gases called Reducing Atmosphere Drying (RAD), showed a protective effect on the nutritional and sensory properties of many fruits, including apples and apricots (Alwazeer, 2018; Alwazeer and Örs, 2019). The above reports revealed that the use of an H₂-incorporated atmosphere in drying fruits allowed the obtaining of dried products with the closest color values (dark-lightness (*L**), green-red (*a**), blue-yellow (*b**) and total color change (ΔE)) to the fresh sample compared with freeze, hot air, and vacuum drying methods. Additionally, The antioxidant activity (DPPH and ABTS) and total phenolic and flavonoid contents were the highest in RAD-dried fruit.

4.2 H₂ and food packaging

Limited research has focused on directly utilizing pure hydrogen (H₂) through the fumigation method as a postharvest treatment. However, Jiang et al. (2021) have studied the postharvest treatment

of Chinese chives with H₂ using fumigation and proved its potential to effectively regulate the quality of fresh produce during refrigeration storage (Jiang et al., 2021). Their findings showed a significant reduction in decay incidence, weight loss ratio, soluble protein content, ROS production, and a decelerated decrease in total phenolic, flavonoid, and vitamin C contents. These positive outcomes are attributed to the upregulated activities of superoxide dismutase, peroxidase, catalase, glutathione reductase, and ascorbate peroxidase.

In food packaging, the inclusion of H₂ in the package atmosphere besides CO₂ and N₂ (RAP) protected the quality attributes of different foods such as strawberries (Alwazeer and Özkan, 2022), cheese (Alwazeer et al., 2020), and fish (Bulut et al., 2023).

Recognized for their high perishability and short shelf life, strawberries were the subject of examination in the study conducted by Alwazeer and Özkan (2022). The strawberries were packaged under two types of reducing atmospheres [RAP1 (5% CO₂, 4% H₂, 91% N₂) and RAP2 (10% CO₂, 4% H₂, 86% N₂)], two modified atmospheres [MAP1 (5% CO₂, 95% N₂) and MAP2 (10% CO₂, 90% N₂)], as well as a control group (air), before being stored for 12 weeks at 4°C. Upon concluding the storage period, RAPs exhibited superior attributes in terms of total soluble solids (TSS), firmness, lightness value (*L**), and green-red chromaticity (*a**), as well as phenolic and anthocyanin contents and antioxidant activity, surpassing the performance of MAP and the control. RAP2 demonstrated a more pronounced effectiveness in preserving freshness indices than RAP1, while MAP2 outperformed MAP1, with RAP2 as the most effective preservation characteristic.

H₂ has also been investigated as a way to enhance the preservation of animal-derived products. The study by Bulut et al. (2023) tested different gas compositions with hydrogen to extend the shelf life of refrigerated rainbow trout. A control group and four distinct treatment samples were employed: Control (air), MAP1 (50% CO₂/50% N₂), MAP2 (60% CO₂/40% N₂), RAP1 (50% CO₂/46% N₂/4% H₂), and RAP2 (60% CO₂/36% N₂/4% H₂). In the control group, lipid oxidation levels surpassed acceptable levels after 5 days, contrasting with treatment samples that consistently remained within acceptable limits throughout the storage period. Similarly, acceptable limits for TVB-N were breached after 7 days in the control group, while the treatment containing H₂ maintained levels below the acceptable limit. The microbial counts in the control group exhibited a gradual increase, indicative of quality deterioration, whereas the samples under the modified atmosphere experienced limited changes. Microbial levels in the treated samples remained below the acceptable limits until the 10th day, whereas the control samples surpassed the limits by the 3rd day of storage.

Alwazeer et al. (2020) conducted a study investigating the utilization of a gaseous mixture containing H₂ for packaging fresh cheese samples. The cheese samples were packaged under different conditions [RAP 1 (90% CO₂/6% N₂/4% H₂), RAP 2 (50% CO₂/46% N₂/4% H₂)], modified atmosphere packaging (MAP) [MAP 1 (90% CO₂/10% N₂), MAP 2 (50% CO₂/50% N₂), and MAP 3 (air)], as well as an unpackaged control, all without the use of any preservatives. Subsequently, the samples were stored at + 4°C for 7 weeks. RAP one exhibited color and titratable acidity values closest

to those of the fresh sample. The total mesophilic-aerobic bacteria levels varied, with the control samples having the highest and the RAP one samples showing the lowest. Over time, yeast mold counts increased in all sample groups, but the RAP groups consistently exhibited the lowest levels. These findings suggest that introducing H₂ either during fumigation or into modified atmospheres holds potential for the agri-food industry, as evidenced by the positive outcomes observed.

4.3 H₂ and food safety

In food safety, the H₂-incorporated atmosphere provided an additional benefit to the product where H₂ exhibited a restrictive effect against the formation of biogenic amines (BAs) in the product, including fish (Sezer et al., 2022), butter (Bulut et al., 2022), minced meat (Çelebi et al., 2024), and red beetroot pickle (Alwazeer et al., 2022a). Another safety concern that was examined was the presence of heavy metals. In the preparation of butter, using HRW in the washing step of cultured raw butter led to a decrease in the content of heavy metals in the product (Alwazeer et al., 2022b). On the other hand, this positive impact of H₂ on reducing the heavy metal content in the product has been also shown in animal studies (Köktürk et al., 2022a; Köktürk et al., 2022b).

5 Hydrogen in the valorization of agri-food waste

Due to the negative perception of synthetic food additives among consumers, food processors have started seeking “green” or “natural” alternatives. Despite the fact that these natural options often fall short of synthetic additives in terms of technological and economic efficiency, they remain popular with consumers who prioritize health and sustainability. Furthermore, global warming and the effects of climate change have further driven the need for sustainable practices. This environmental shift has promoted the valorization of underestimated agri-food wastes, decreased crop production, and ultimately increased crop prices. Consequently, the food industry is increasingly motivated to find innovative solutions that align with both consumer preferences and environmental sustainability. To satisfy the consumer, food processors are trying to find an economical source of these “natural” ingredients for additives in the food industry. Importantly, agri-food wastes have been the most attractive sources of “natural” additives due to their low price and high availability.

An extraction process is required to separate phytochemicals from plant materials. Despite the development of many extraction methods, conventional methods like maceration are still used widely due to their low cost and infrastructure demands. Alwazeer’s team has recently developed a hydrogen extraction method by modifying the traditional maceration method. The hydrogen extraction method depends on the infusion of hydrogen gas into the solvent before (or during) immersion of the plant material. This method was examined on different agri-food wastes, showing encouraging results. The extraction of some phytochemicals such as phenolics, flavonoids, anthocyanins, and other antioxidants was enhanced

when hydrogen-rich solvent was used instead of pure solvent. The extraction yield of phytochemicals improved for different solvents, including water, ethanol, methanol, and *n*-hexane. The safe installation of the hydrogen extraction method is possible at the laboratory or plant scale (Alwazeer, 2023).

The amounts of certain flavonoids and non-flavonoids were increased several times when HRW was used as a solvent in the extraction of phytochemicals from different agri-food wastes and by-products, including tomato peels, apple peels, lemon peels, cabbage, and carrots (Alwazeer and Elnasanelkasim, 2023). Similar results could be obtained for by-products such as olive leaves when two types of HRW were tested: hydrogen bubbling (H_2 water) and magnesium-water reaction (Mg water) (Alwazeer et al., 2023d). Hydrogen-rich solvent (water, ethanol, methanol) was also evaluated for extracting phytochemicals from red beetroot (Alwazeer et al., 2023b). Moreover, the incorporation of hydrogen into polar and non-polar solvents for extraction of phytochemicals from a fatty by-product, i.e., olive pomace oil, was also examined (Ceylan et al., 2023). Results showed that the incorporation of hydrogen into both methanol and *n*-hexane led to improved extraction of many phytochemicals, including phenolics, flavonoids, and antioxidants. Similarly, the hydrogen-rich solvent extraction method was evaluated to extract bioactive compounds from another by-product, i.e., propolis (Yurt, 2023). Incorporating hydrogen into water, methanol, and ethanol could yield higher phenolics, flavonoids, anthocyanins, and antioxidants. Hydrogen-rich solvent extraction method was also studied to compare its efficiency for extracting phytochemicals from agri-food waste, i.e., lemon peel, against the effect of temperature (Alwazeer et al., 2023c). The authors report that the hydrogen incorporation into solvents (water, ethanol, and methanol) showed the highest levels of phenolics and flavonoids in hydrogen-rich methanol, followed by hydrogen-rich ethanol at both 25°C and 35°C, with the highest phytochemical extraction found for hydrogen-rich methanol at 35°C. The study concluded that incorporating hydrogen into solvent was more efficient for extracting phytochemicals than increasing the temperature from 25°C to 35°C.

This novel extraction method of hydrogen-rich solvent was compared with the widely used emerging method, i.e., supercritical fluid extraction. Herein, the hydrogen extraction method was more potent for extracting all phytochemical groups from all studied agri-food wastes, i.e., tomato peel, green apple peel, lemon peel, orange carrot, and red cabbage. The hydrogen extraction method could also extract phenolic acids and flavonoids better than supercritical fluid extraction (Alwazeer et al., 2023a). A recently published review discussed the possible mechanisms behind this potent extractability of hydrogen-rich solvent (Alwazeer, 2023). Ryu et al. (2019) examined the superiority of the hydrogen extraction method for phenolic extraction from green tea leaves compared with other gases, including N_2 , CO_2 , and air. They found that the physical and morphological modification in the softness and flexibility of the surface of the plant material, examined by scanning electron microscopy, occurred in H_2 -treated green tea leaves. The hydrogen extraction method was described as being simplistic, low cost, requiring less additional equipment and low energy consumption, non-toxic, and sustainable (Alwazeer, 2024b).

6 Possible mechanisms of hydrogen action

The impact of hydrogen on various biological systems, including animals and plants, has prompted researchers to investigate its potential effects and underlying mechanisms. Despite the many effects of H_2 treatment in plants, the exact molecular mechanisms by which H_2 acts on plant cells have not been convincingly reported (Table 4). Several mechanisms of H_2 action in cells have been mooted, although not supported by experimental data, including acting as a redox couple or directly interacting with hydrophobic pockets in proteins, which has been reviewed recently (Hancock et al., 2022).

6.1 Fe-porphyrin-mediated scavenging of hydroxyl radicals

There are several potential modes of action of H_2 in cells. In their 2007 paper, Ohsawa et al. (2007) showed that H_2 could remove $\bullet OH$ from solution, but that H_2 had no effect on some other reactive signaling molecules such as H_2O_2 or $NO\bullet$ (Ohsawa et al., 2007). As $\bullet OH$ can act as a cellular signal (Richards et al., 2015), this could account for some of the effects seen. Indeed, many reports have been published showing that the antioxidant capacity of cells increases during H_2 treatment, such as during salt stress (Xu et al., 2013; Fu et al., 2020) and nitrogen deficiency (Jiang et al., 2023).

As stated, one of the seminal papers on H_2 action was by Ohsawa et al., in 2007, albeit in animal cells (Ohsawa et al., 2007). Subsequent papers have highlighted the role of heme in this process of hydroxyl radical removal. For example, Kim et al. (2022) suggested a mechanism whereby H_2 reacts with heme, with the resultant products mediating the scavenging of $\bullet OH$. More recently, a paper has suggested a similar mechanism (Jin et al., 2023). Here, authors identified that Fe-porphyrin is a target for H_2 and that Fe-porphyrin in the free or protein-bound state can use H_2 to scavenge hydroxyl radicals. This is a very significant result, as it could explain many of the downstream effects of H_2 . To illustrate, such a mechanism could lead to the activation of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), as suggested by Ohta (2023), which is known to be involved in oxidative stress responses (Yu et al., 2021) and involves a basic leucine zipper (bZIP) transcription factor. Also of significance in the Jin et al. (2023) paper, it shows that the interaction of H_2 and porphyrin can lead to CO_2 being converted to CO, and therefore, they suggest that H_2 is upstream in CO signaling. This is also interesting as it is known that an enzyme linked to CO metabolism, i.e., heme oxygenase, is a target for H_2 effects (Shen et al., 2017).

Despite findings of interactions between H_2 and Fe-porphyrin molecules, it is likely that other mechanisms are also at play in order to account for all effects seen in all organisms for which H_2 responses have been reported. It is also unlikely that the Fe-porphyrin-mediated scavenging role of H_2 would account for all effects seen when, for example, the localization of the production of $\bullet OH$ and the diffusion of H_2 from outside the cells - as it is often applied exogenously - are considered. Therefore, other mechanisms of H_2 action, such as Fe-porphyrin-mediated CO production or other non-Fe-porphyrin mechanisms, are likely, and several mechanisms are probably being enacted simultaneously.

TABLE 4 Proposed mechanisms of hydrogen's action in plant cells.

Proposed mechanism	Comment	Reference(s)
Scavenging of hydroxyl radicals	Other ROS not scavenged	Ohsawa et al. (2007)
Interaction with Fe-porphyrin	May explain many effects and only target with experimental data	Jin et al. (2023)
Scavenging ROS through a heme-mediated mechanism	May explain some localized effects; only computational data	Kim et al. (2022); Ohta (2023)
Effects mediated by HO-1	Would explain some effects	Shen et al. (2017)
Redox effects	No direct evidence outside of bacterial systems	Peck (1959); Hancock et al. (2021)
Spin state effects	No direct evidence for this	Hancock and Hancock (2018)
Acting through protein pockets	Globin proteins are a good model system, as seen with "Xe pockets"	Hancock et al. (2022); Alwazeer et al. (2023b); Hancock (2023)

6.2 H₂ modulates the action of heme-oxygenase

One suggested mechanism is that H₂ modulates the action of heme-oxygenase, as mentioned above, particularly HO-1 (e.g., Shen et al. (2017)). This would then involve mediating effects by CO, another gas known to have cellular effects (Stucki and Stahl, 2020). However, how H₂ would directly influence the activity of HO-1 is unclear. Unlike ROS and RNS, there is unlikely to be a covalent modification of proteins provoked by H₂, in comparison to oxidation (by ROS) (Turell et al., 2020) and S-nitrosylation (by RNS) (Feng et al., 2019). This applies to the control of any protein, not just HO-1. The unlikelihood of direct protein modifications by H₂ further underscores the attractiveness of the Fe-porphyrin molecule as a target of H₂ because such interaction does lead to the production of CO (Jin et al., 2023).

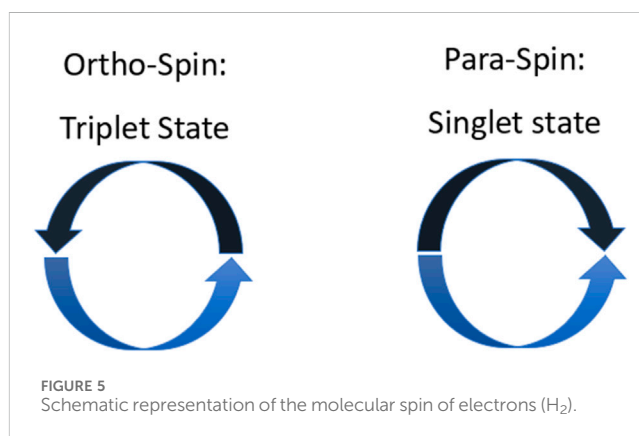
6.3 H₂ effects through its redox action

Some proteins rely on their function by having prosthetic groups, and such mechanisms often rely on changes in the redox state of such non-protein groups. A typical example would be the heme prosthetic of globins, cytochromes, etc. H₂ can undergo redox reactions itself, and it has been reported that such action can alter the redox poise of Fe in heme groups in bacterial cytochromes, such as cytochrome c₃ (Peck, 1959). Although such redox activity was ruled out as accounting for H₂ action by others (Ohsawa et al., 2007), not all possible redox interactions have been studied, especially in plants. Therefore, it is too early to rule this out completely (Hancock et al., 2021).

6.4 The molecular spin effects of hydrogen electrons

A proposed, though unproven, mechanism of H₂ in biological systems involves its different spin states (Hancock and Hancock, 2018) (Figure 5).

H₂ can potentially alter the electron configuration of other molecules, particularly radicals. Thermal polarization favors the



triplet (ortho) state, with ~75% of H₂ molecules in this state at room temperature (O'Neill et al., 2023). The ortho spin generates a weak diamagnetic force (Tarzad, 2020), making it unlikely to affect ROS formation directly via transition metal reduction.

The singlet state of H₂ may not directly form radical pairs but may have active redox potential through interactions with metalloproteins. Radical pair formation occurs when forward electron translocation is inhibited (e.g., Complex 1, mitochondria), and this contributes to ROS formation (Hore and Mouritsen, 2016). For example, A flavin-superoxide pair with a singlet spin forms F + H₂O₂, while the triplet state disassociates into free radicals FH[•] and O₂^{•-} (Ramsay and Kattnig, 2022). Should H₂ interact with transition metals components of proteins, as suggested by Hancock and Hancock (2018), Kim et al. (2022), Jin et al. (2023), and Ohta (2023), with electrons in the antiparallel configuration, it is possible that the metal group would more easily catalyze the reduction of radicals via reducing the disassociation energy of free H₂ (~4.64 eV--~2.35 eV). Logically, the H₂ radicals could react with radical oxidants such as O₂^{•-} and [•]OH, reducing the pro-oxidation status of cells, but potentially also biologically important radicals including NO[•], etc. Therefore, the para (singlet) state, of H₂, though less prevalent, may play a significant role, especially in cooler soil temperatures. However, as mentioned previously, this would be regulated by the spatial and temporal availability of the perceived radicals and H₂.

Due to the weak diamagnetic forces at play, the triplet state of H₂ may influence cellular activities such as cell signaling, gene regulation, and metabolism by stabilizing the protein structure (protein pocket theory) (Hancock et al., 2022; Alwazeer et al., 2023b). H₂ might mediate signaling by affecting protein phosphorylation, depending on the protein's temporal configuration. Hypothetically, while the singlet state molecule could influence redox balance, and the triplet state may support protein function, there is currently no evidence to support this, and the only evidence-based target of H₂ remains the Ferroporphyrin target.

6.5 H₂ interactions with protein pockets

Regardless of the mechanism(s) used by H₂ to bring about biological effects, they have to account for several phenomena. H₂ needs to be able to be moved from its administration location (treatment) to the site of action. It needs to initiate a response that is probably far greater than the H₂ concentration sensed by the cell, and the response has to last longer than the possible stability of the H₂ gas in the solution. Therefore, having a scavenging mechanism where the removal of •OH seems unlikely to be the sole way H₂ acts. Although hydroxyl radicals do have a signaling role in plants (Richards et al., 2015), it is probably far less so than other ROS or RNS, many of which appear to have a far greater significance when it comes to signaling, such as NO• and H₂O₂, whilst neither are reported to be affected by H₂ (Ohsawa et al., 2007).

A possible mechanism that could account for many of the phenomena seen when adding H₂ to biological systems would be if H₂ interacted directly with proteins. In the globin family of proteins, for example, it is known that inert gases can interact with cavities within protein structures. It has even been shown that these gaseous atoms are able to migrate into proteins through cavity structures (Turan et al., 2023). Other inert gases, such as argon, are also known to have biological effects (Ye et al., 2013). It is, therefore, not a major leap of thought that a small, relatively inert molecule such as H₂ could also partake in such interactions with proteins (Hancock et al., 2022). More recently, it has also been suggested that such mechanisms may account for some of the effects seen in the presence of ROS and RNS (Hancock, 2023). Therefore, this may be a far more widespread mechanism for the control of protein function than previously thought. H₂ being involved here would make much sense and would account for how H₂ has such a wide range of effects, can be moved around organisms, and can have effects far after any treatment given.

The mechanism(s) of H₂ action also need to account for the plethora of effects seen in plants through many developmental stages, from seed to flowers to fruits, as well as a range of stress responses, from salinity (Yu et al., 2021), heavy metal tolerance (Cui et al., 2014) to drought tolerance (Zhao G. et al., 2021). Therefore, there is almost certainly more than one pathway in which H₂ is involved. This makes it more challenging to elucidate, but it could account for the range of effects seen, from being favorable for plants to being able to have therapeutic effects in humans.

7 Regulatory, toxicity, and safety of hydrogen utilization in crop and food production

7.1 Regulations of H₂ use

The European Commission has approved hydrogen gas for use in foods and beverages as a food additive with an E949 code for infants and young children at *the quantum satis* maximum level (Alwazeer, 2024a). Although the effects of H₂ are safe (European Commission, 2011; U.S. Food and Drug Administration, 2023), there could be detrimental (or beneficial) effects when H₂-producing ingredients are used, whilst some by-products could be harmful. For example, metallic minerals may raise the pH of the water too high or contribute to osmotic stress in the plants. Some ingredients, such as aluminum and other metal hydrides, could be damaging. Additionally, the infusion of pure H₂ gas into water sparges out dissolved oxygen gas in the water. Removal of O₂ gas could be helpful in certain food preservation and packaging to reduce oxidation; however, its reduction in agricultural water may prove harmful to the plants, which may account for some of the decreased benefits of H₂ at higher concentrations.

7.2 Toxicity of H₂

The application of H₂ as a gas (and therefore, by extension as HRW) appears to be inherently non-toxic and safe for biological materials, as exemplified by its use as a high-pressure gas for divers, which has been used for approximately 80 years (Bjurstedt and Severin, 1962). No ill effects of H₂ treatment have been reported for humans, and high concentrations are tolerated as long as oxygen is present, for example, with the use of oxyhydrogen (Russell et al., 2021). However, a high concentration of H₂ might have a negative effect on plant growth. Geologists documented that the lands in Brazil, Namibia, and Australia that leak hydrogen are curiously devoid of vegetation (Hand, 2023). Additionally, several studies observed that high concentrations of H₂ showed reduced benefits in certain plants, which might be attributed to the different sensitivity of different plants to H₂ (Li et al., 2021b). Therefore, the dosage of H₂ should be considered in agricultural applications.

7.3 Safety of H₂ use

Proper handling precautions and procedures are essential due to hydrogen's flammability and potential for explosive reactions, i.e., 4%–75% and 18.3%–59% vol with air (Alwazeer, 2019). For instance, hydrogen must be stored in appropriate cylinders that are kept in well-ventilated areas, away from heat sources, ignition, and direct sunlight (Abohamzeh et al., 2021).

Given that H₂ is lighter than air, ensuring proper airflow ventilation is necessary to prevent its accumulation, which could lead to potentially explosive conditions. Equipment, fittings, and connections using H₂ should be equipped with detectors and monitors that can provide early warnings of malfunctions and potential leaks. Pressure regulators and flow meters are also essential, as they can detect sudden pressure changes and help

prevent accidents related to faults in H₂-associated equipment. It is also important to ensure that individuals working with hydrogen are thoroughly trained in safe handling, storage, and emergency procedures. For example, workers should be familiar with evacuation protocols and how to handle fires safely. Regular drills and continuous education on the latest safety standards and technologies can further enhance workplace safety. Proper maintenance and adherence to safety guidelines will ensure that hydrogen can be used effectively and safely in food production and processing environments.

8 The current status, costs, challenges, and future prospects of hydrogen-based agriculture and economic prospects

8.1 Current status of H₂

The observations and studies discussed so far suggest that H₂ is a promising solution to address challenges in agricultural sustainability. With the global agricultural sector facing issues such as soil degradation, water scarcity, and environmental degradation due to chemical inputs, the adoption of H₂-based strategies offers a promising avenue for positive change. For example, by leveraging H₂ fertilization techniques, which promote the proliferation of plant-growth-promoting rhizobacteria and enhance nutrient uptake efficiency, farmers can mitigate the reliance on chemical fertilizers while maintaining or improving crop yield and quality (Li et al., 2021b). Furthermore, the integration of HRW irrigation systems holds the potential to enhance nutritional food quality by increasing the content of beneficial phytochemicals in crops, vegetables, and fruits while simultaneously reducing heavy metal accumulation, among other benefits (Wang et al., 2019; Yao et al., 2024).

8.2 Examples of wide-scale use of H₂ in agriculture

The application of hydrogen in agriculture has recently started at an economical scale. In China, the field trials of H₂-based irrigation are being carried out gradually. For example, Air Liquide and Nanjing Agricultural University established Hydrogen Agricultural Bases for strawberries and tomatoes. It reported an increase in tomato yield by ~39% and ~28% in the absence and presence of fertilizers, respectively, by H₂-based irrigation (Li et al., 2024). The flavor of strawberries (Li F. et al., 2022) and the nutrient quality of tomatoes (Li et al., 2024) were also improved. The HRW-irrigated strawberries cost up to about 42 USD/kg, while the regular ones are priced at 8–11 USD/kg. Consistently, the yield of Chinese cabbage was increased by 32.7% after HRW irrigation (Liu et al., 2024). Moreover, a good harvest of the HRW-irrigated rice was achieved in the demonstration fields in Shanghai Qingpu District (9.67 ha, an 18.8% increase in yield, in 2022) and Songjiang District (5.33 ha, a 6.9% increase in yield, in 2023), which were widely reported in the media. Based on these results, the rice planting area with HRW irrigation in Songjiang will be expanded to 26.67 ha by

2024. As for the large-scale promotion of H₂-based irrigation in rice, fruits, and vegetables, the cost is the main bottleneck. At present, it may cost at least 5.5 USD to inject enough H₂ into 0.067 ha mainly due to the equipment and transportation. Since the prospects of H₂-based irrigation in increasing the added value of agricultural products are encouraging, governments, enterprises, and scientific research institutions may join forces to overcome difficulties and drive sustainable agriculture.

8.3 Costs of H₂ use

With the rapid development of the H₂ energy industry, the cost of H₂ production is experiencing a significant decline. Currently, more than 95% of commercially produced H₂ relies on fossil fuels, priced at 1–2 USD/kg H₂ in the United States, Europe, and China, while the cost of green H₂ produced through water electrolysis is 5–10 USD/kg H₂ (Finke et al., 2021; Pivetta et al., 2022). Scaling up in wind power and photovoltaic technologies is expected to lead to a 30%–80% reduction in the cost of electrolytic H₂ production by 2050, bringing it closer to the cost of H₂ production from fossil fuels (Finke et al., 2021). Considering the limited consumption of H₂, the cost of H₂-based food/crop production primarily depends on equipment and labor expenses. Therefore, the technology of H₂ supplementation in the agriculture and food industry emerges as both economically viable and practicable.

8.4 Challenges of H₂ use

Nevertheless, despite the promising benefits of H₂-based agricultural solutions, several challenges need to be addressed to ensure widespread adoption and effectiveness. These challenges include technological readiness, scalability, and regulatory frameworks governing the use of novel agricultural inputs. As discussed, H₂ is a gaseous molecule that does not remain in solution indefinitely; thus, point-of-use H₂ irrigation needs to be developed to ensure H₂ delivery. Such devices are not readily available in the marketplace and thus need to be engineered, especially for large-scale applications. Although we are unaware of any governmental restrictions on molecular hydrogen for agricultural use, different countries may have different approval requirements.

8.5 Future prospects of H₂ use in agriculture and food industry

The level of readiness by large-scale farmers to implement hydrogen-based technologies is unknown as there are still many questions that need to be addressed. It is still unclear what method is the most effective, if H₂ should be applied to every part of the process, and what the exact cost-benefit analysis is on each possible application and step individually and combined. However, this hasn't prevented some groups from implementing hydrogen technologies when convenient. The authors are aware of several smaller agricultural outlets in Asia, EU, and United States as well as individuals across the globe with personal gardens/farms that have

incorporated H₂ in agriculture. At this point, it is clear from the published research that using molecular hydrogen does not impair or reduce crop yields or have other negative effects. Thus, these early adopters are likely to only experience potential benefits, which, over time, should address the cost/benefit analysis that will ultimately pave the way for larger corporations to determine the viability of H₂-based agriculture. This will allow stakeholders and engineers to identify key barriers to implementation and develop strategies to overcome challenges to unlock the full potential of molecular hydrogen in promoting agricultural sustainability.

9 Conclusion

In conclusion, the multifaceted potential of molecular hydrogen as a 'green' solution extends far beyond addressing energy needs. The critical need for sustainable solutions is paramount in the face of global challenges such as climate change, communicable disease epidemics, and a growing population. The versatile applications of H₂ in health, agriculture, and the food industry position it as a uniquely suitable approach to address today's significant challenges, fostering abundant crop production and positively impacting the agri-food chain. Hydrogen's role as a green alternative fertilizer is underscored by its ability to promote plant growth, enhance nutrient uptake, and reduce reliance on chemical fertilizers. Although there have been discussions about using H₂ in agriculture, no reports about its potential application in the wider food industry are available in the literature.

Applying hydrogen-rich water in irrigation can offer health-related benefits by mitigating heavy metal accumulation in crops. In the food industry, adopting hydrogen-based strategies may be instrumental in preserving nutritional and sensory properties, reducing heavy metal content, limiting biogenic amine formation, increasing microbiological safety, and extending product shelf life. Embracing hydrogen within a sustainable development framework can ensure the production of safe, high-quality products, fostering a holistic approach from the field to the fork. While, it is important to consider the challenges and costs associated with implementing hydrogen-based solutions on a large scale, the potential benefits are significant. Further research and development are also necessary to optimize the hydrogen application methods according to the specifications of each crop/product and to ensure they are economically viable and widely accessible to farmers and food producers.

Author contributions

DA: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Software,

Supervision, Visualization, Writing–original draft, Writing–review and editing. JH: Conceptualization, Data curation, Investigation, Methodology, Resources, Software, Visualization, Writing–original draft, Writing–review and editing. GR: Conceptualization, Data curation, Investigation, Resources, Software, Visualization, Writing–original draft, Writing–review and editing. AS: Conceptualization, Data curation, Investigation, Resources, Software, Visualization, Writing–original draft, Writing–review and editing. LL: Conceptualization, Data curation, Investigation, Resources, Software, Validation, Visualization, Writing–original draft, Writing–review and editing. AÇ: Investigation, Writing–original draft. TE: Data curation, Investigation, Writing–original draft. TL: Conceptualization, Data curation, Investigation, Resources, Software, Validation, Visualization, Writing–original draft, Writing–review and editing.

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Conflict of interest

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