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Molecular hydrogen content of different dietary supplements

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Abstract: The main goal of this study was to evaluate the concentration and release dynamics of molecular hydrogen (H_2, dihydrogen) in dietary evaluate supplements and identify products that provide a biologically significant amount of dihydrogen suitable for human consumption. We examined ten commercial supplements marketed for their dihydrogen content, including slow-release capsules and tablets (4 products), effervescent powders and tablets (5 products), and canned ready-to-drink beverage (1 product). These products were acquired either through online purchases, from retail stores, or obtained free of charge directly from the manufacturers upon request. Dihydrogen concentration was measured using a highly sensitive Clark-type hydrogen microsensor with a detection limit 0.05 μmol·L^{-1}. Out of the ten products examined, only three (30.0%) exhibited dihydrogen levels surpassing the levels marketed as biologically relevant (500 μmol·L^{-1}), and one of these products (a canned ready-to-drink beverage) approached this level with a concentration of 439.2 μmol·L^{-1}. Interestingly, all slow-release capsules yielded negligible amounts of hydrogen (< 2 μmol·L^{-1}), while a slow-release tablet delivered 43.6 μmol·L^{-1} of dihydrogen per single dose. The substantial variance in dihydrogen content among the assessed supplements holds significant implications for the general public, as high-potency products have the potential to provide up to 7 000 times more dihydrogen per single dosage compared to their low-potency counterparts.

Keywords: dihydrogen; electrochemical; food supplements; slow-release

Molecular hydrogen (H_2, dihydrogen) is an innovative bioactive non-nutrient with diverse applications in experimental and clinical nutrition (Ostojic 2019; Artamonov et al. 2023; Ramanathan et al. 2023; Todorovic et al. 2023; Xie et al. 2023). Dihydrogen is commonly available in various dietary supplements, including ready-to-consume liquid solutions, capsules, orally disintegrating tablets, and effervescent powders and tablets (Ohta 2014). Many manufacturers of dihydrogen supplements anecdotally claim that their products are highly saturated with gaseous hydrogen, with concentrations exceeding 500 μmol·L^{-1} [~ 1 ppm (parts per million)], a threshold often considered critical for the biological effects of dihydrogen. However, human studies not revealed exact concentrations (mass per unit volume) required for the biological effects of dihydrogen in human nutrition. The only standard proposed thus far is by the International Hydrogen Standards Association (IHSA, http://www.intlhsa.org), indicating a minimum concentration of 250 μmol·L^{-1}. Nonetheless, it remains unclear whether supplements available on the market actually deliver a dihydrogen concentra-

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tion surpassing the IHSA target (250 μmol·L^{-1}) or levels marketed as biologically relevant (500 μmol·L^{-1}). There is currently no study that has analysed the dihydrogen content across supplements using rigorous methods measuring its levels, especially for so called 'slow-release' dihydrogen formulations. Traditional methods of measuring dihydrogen in final products are often qualitative, and do not measure the actual levels but instead focus on indicators like oxidation-reduction potential (ORP) and pH (Ohta 2015; LeBaron and Sharpe 2022). Hence, the primary objective of this study was to assess the concentration and release dynamics of dihydrogen in various supplements using a highly sensitive electrochemical method, and identify products that offer a biologically relevant amount of dihydrogen for human consumption.

MATERIAL AND METHODS

Ten commercial supplements (n = 10) marketed for dihydrogen content were analysed in this study. The products were either purchased online or from retail stores, or obtained at no cost from manufactures upon request. The supplements were produced by companies from various countries, including Canada (1 product), Czechia (1 product), Japan (4 products), Serbia (1 product), the United Kingdom (1 product), and the United States of America (2 products). The products evaluated for dihydrogen content included slow-release capsules and tablets (4 products), effervescent powders and tablets (5 products), and canned ready-to-drink beverage (1 product). Table 1 illustrates readily available information about the products.

Dihydrogen concentration was measured using a highly sensitive Clark-type hydrogen microsensor with a detection limit 0.05 μmol·L^{-1} (Unisense A/S, Denmark). The system was calibrated with a standardised gas mixture (Messer Technogas AD, Serbia) and washed before each test. All supplements were prepared according to the manufacturers' instructions for human consumption, and dihydrogen levels were measured in the ready-to-consume final products (250 mL water solution, temperature of 21 °C). Real-time data for dihydrogen production were monitored until a pill or powder was fully dissolved, or after opening a can for the ready-to-drink beverage, with peak dihydrogen levels recorded. Once the release of dihydrogen from the water solution had plateaued (and balance with dihydrogen peak levels were established), any residual dihydrogen was removed by stirring the experimental solution with a magnetic stirrer. To simulate the effect of stomach acid on the release of dihydrogen in slow-release products, the solution was further adjusted to pH 2 by mixing it with hydrochloric acid (HCl, 1 : 1) after 300 s. Each product was tested twice, and the mean values of dihydrogen levels were presented. The study was conducted at Applied Bioenergetics Lab at the University of Novi Sad between March and October 2023. All procedures were performed in accordance with the principles of Good Laboratory Practice, and rigorous ethical and scientific standards. The study was registered at the institutional trial registry (# H-UNS-11-23).

Table 1. Label information for supplements included in the analysis

Product	Country	Dosage form	Serving size	Ingredients	Dihydrogen/serving (μmol)*
1	UK	RTD beverage	440 mL can	water, Ca, Mg, Na, HCO_3^-, K	2 480.6
2	Japan	SR capsule	3 caps	Ca, Mg	–
3	Japan	SR capsule	3 caps	Ca, Mg	–
4	Japan	SR capsule	3 caps	mineral blend	–
5	Czechia	SR tablet	1–3 tabs	Ca, Mg, maltodextrin	496.1
6	USA	EFF tablet	1 tab	mineral blend	–
7	USA	EFF tablet	2 tabs	Ca, P, Mg, mineral blend	–
8	Canada	EFF tablet	1 tab	Mg	3 969.0
9	Japan	EFF powder	1 sachet	unknown	3 372.0
10	Serbia	EFF powder	1 packet	polynutrient complex†	496.1

* Indicates the dosage of dihydrogen per serving, as calculated from the parts per million (ppm) declared on the label or promotional materials; † contains carbohydrate, dietary fiber, sugar alcohol, *L*-leucine, *L*-isoleucine, *L*-valine, calcium, magnesium, vitamin C, and citric acid; RTD – ready-to-drink; SR – slow-release; EFF – effervescent; Ca – calcium; Mg – magnesium; Na – sodium; K – potassium; P – phosphorus; HCO_3^- – bicarbonate

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RESULTS AND DISCUSSION

The labeled dihydrogen content per serving of the five products ranged from approximately 500 to 4 000 µmol, but the manufacturers did not provide any information about whether the concentration of dihydrogen is expressed per serving size (which varies considerably among products) or per litre of the water solution. Notably, five out of ten supplements (50.0%) did not provide any information regarding dihydrogen levels on the product label. The peak concentrations of dihydrogen and dihydrogen release dynamics per single dosage for all ten supplements are depicted in Figure 1. Only three out of ten products (30.0%) contained dihydrogen levels exceeding the threshold marketed as biologically relevant of 500 µmol·L^{-1}, with one product (canned ready-to-drink beverage) neared this level (439.2 µmol·L^{-1}). These products included two effervescent powders manufactured in Japan and Serbia, and one effervescent tablet made in Canada. The time for those products to reach the critical threshold of 500 µmol·L^{-1} in the final solution varied from approximately 300 to up to 800 s. Above four products surpassed the IHSA target of 250 µmol·L^{-1}. Interestingly, all slow-release capsules yielded negligible amounts of hydrogen (< 2 µmol·L^{-1}), while a slow-release tablet delivered 43.6 µmol·L^{-1} of dihydrogen per single dose.

For the first time, our report delved into the dihydrogen content of various 'hydrogen-rich' dietary supplements accessible in the global market. We discovered that a substantial portion of the supplements assessed in our study contained or generated amounts of hydrogen per single dose that appeared biologically inconsequential (below 250 or 500 µmol·L^{-1}). This raises concerns about misleading consumers who seek effective products. An alarming discrepancy was observed between the declared and actual dihydrogen content, with several products overstating the dihydrogen concentration on their labels by as much as tenfold. Notably, one product containing effervescent tablets and two effervescent powders examined in our study seemed to meet the threshold marketed

(A)

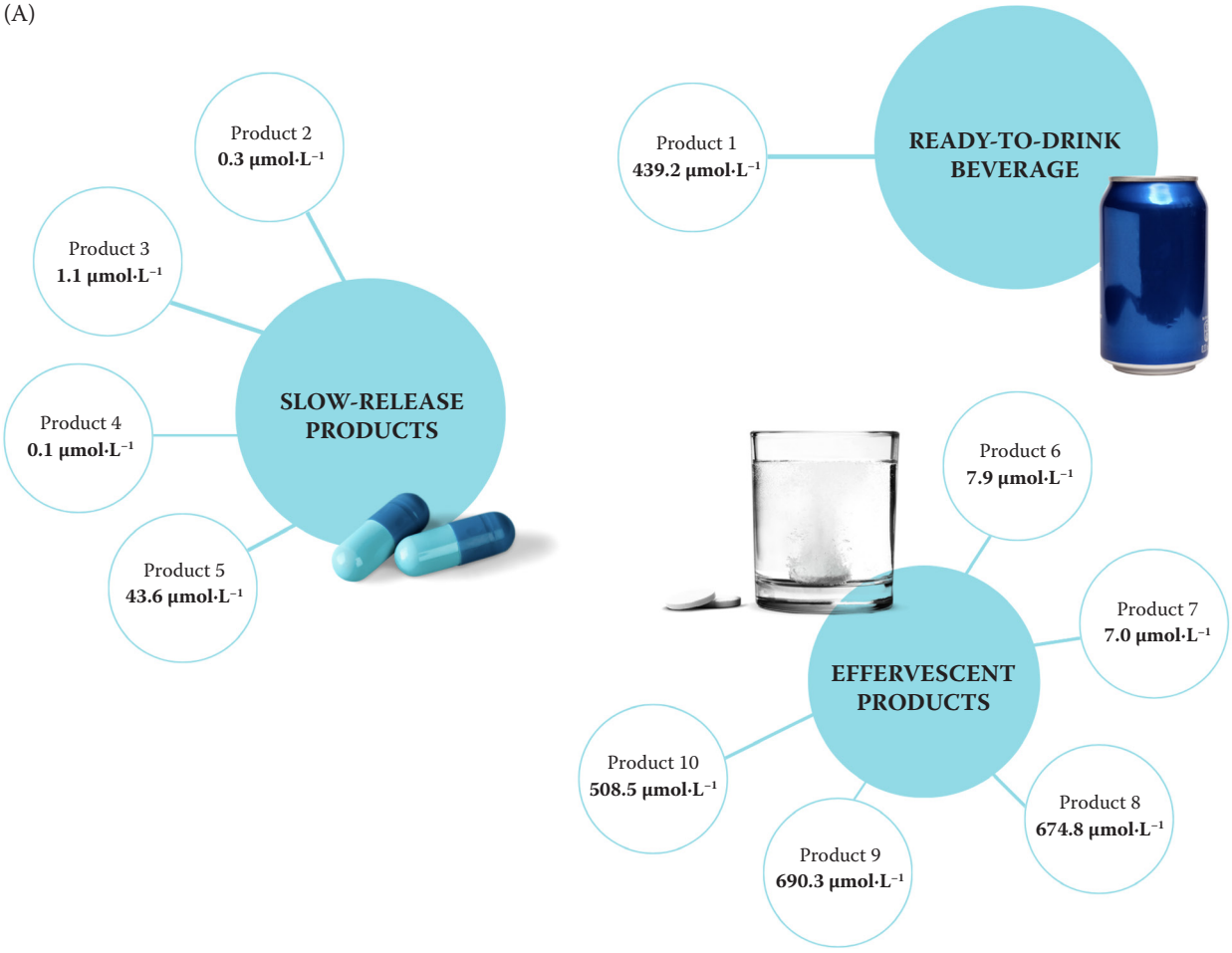

Figure 1. (A) Peak dihydrogen concentrations and (B) dihydrogen release dynamics in ten different 'hydrogen-rich' products

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(B)

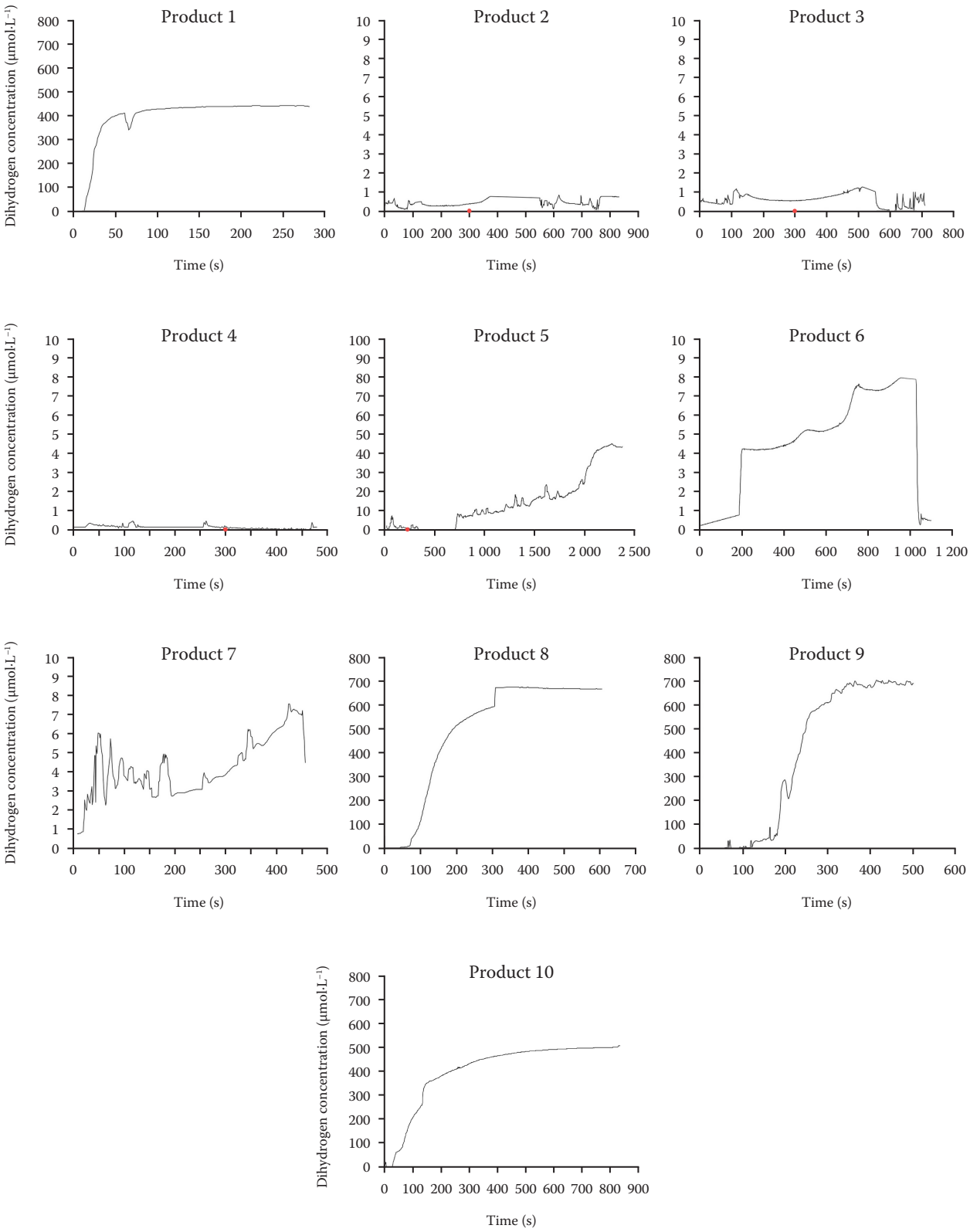

Figure 1. To be continued

Red dots indicate when hydrochloric acid (HCl) was added to the water solution to simulate the effect of stomach acid on the release of dihydrogen in slow-release products

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as biologically relevant for dihydrogen concentrations (> 500 μmol·L^{-1}). However, it's important to note that some of these products still overstated the labeled levels. It's worth noting that few products claimed levels of dihydrogen above the upper level of dissolution in water (793.8 μmol·L^{-1}) under 1 atmospheric pressure at room temperature (Ohta 2014). The wide range of dihydrogen content among analysed supplements is of paramount importance for the general public, as high-potency products could potentially deliver up to 7 000 times more dihydrogen per single serving than their low-potency counterparts.

Several recent studies have identified similar trends, showcasing considerable variability in active components within dietary supplements (LeBlanc et al. 2013; Navarro et al. 2019). The high incidence of mislabelling necessitates more stringent regulation of 'hydrogen-rich' supplements. This might involve mandating the disclosure of dihydrogen content, verified by certified laboratories and methods. Moreover, instances of false or misleading claims about dihydrogen content in dietary supplements might warrant legal action by government authorities. Future research should expand the analysis to other commercially available supplements and various dosage forms containing hydrogen, such as syrups, elixirs, emulsions, lozenges, pastes, decoctions, eye drops, nebulisers, creams, and gels. Additionally, alternative highly sensitive methods for dihydrogen analyses, like high-sensitive gas chromatography, could be considered for more accurate measurements. The Clark-type hydrogen microsensor utilised in our study does not permit the measurement of dihydrogen in micro/macro bubbles that are not dissolved in the solution; this limitation could result in an underestimation of the dihydrogen concentration. Furthermore, our study did not assess the total dihydrogen produced per hydrogen-generating product, and the rate of dihydrogen production may be lower than the rate of molecular hydrogen escaping the solution, leading to a significant loss of dihydrogen per product. Additionally, some products may require more time to produce dihydrogen after consumption, resulting in a larger area under the curve and necessitating a longer monitoring period in a biological system. Finally, the use of different volumes and diameters of beakers to assess dihydrogen concentration in water solutions can influence the dissolution ratio of dihydrogen; this factor should be considered in future studies.

REFERENCES

Artamonov M.Y., Martusevich A.K., Pyatakovich F.A., Minenko I.A., Dlin S.V., LeBaron T.W. (2023): Molecular hydrogen: From molecular effects to stem cells management and tissue regeneration. Antioxidants, 12: 636.

LeBaron T.W., Sharpe R. (2022): ORP should not be used to estimate or compare concentrations of aqueous H_2: An *in silico* analysis and narrative synopsis. Frontiers in Food Science and Technology, 2: 1007001.

LeBlanc E.S., Perrin N., Johnson J.D. Jr., Ballatore A., Hillier T. (2013): Over-the-counter and compounded vitamin D: Is potency what we expect? JAMA Internal Medicine, 173: 585–586.

Navarro V., Avula B., Khan I., Verma M., Seeff L., Serrano J., Stolz A., Fontana R., Ahmad J. (2019): The contents of herbal and dietary supplements implicated in liver injury in the United States are frequently mislabeled. Hepatology Communications, 3: 792–794.

Ohta S. (2014): Molecular hydrogen as a preventive and therapeutic medical gas: Initiation, development and potential of hydrogen medicine. Pharmacology & Therapeutics, 144: 1–11.

Ohta S. (2015): Molecular hydrogen as a novel antioxidant: Overview of the advantages of hydrogen for medical applications. Methods in Enzymology, 555: 289–317.

Ostojic S.M. (2019): Are there natural spring waters rich in molecular hydrogen? Trends in Food Science and Technology, 90: 157.

Ramanathan D., Huang L., Wilson T., Boling W. (2023): Molecular hydrogen therapy for neurological diseases: A review of current evidence. Medical Gas Research, 13: 94–98.

Todorovic N., Fernández-Landa J., Santibañez A., Kura B., Stajer V., Korovljev D., Ostojic S.M. (2023): The effects of hydrogen-rich water on blood lipid profiles in clinical populations: A systematic review and meta-analysis. Pharmaceuticals, 16: 142.

Xie F., Song Y., Yi Y., Jiang X., Ma S., Ma C., Li J., Zhanghuang Z., Liu M., Zhao P., Ma X. (2023): Therapeutic potential of molecular hydrogen in metabolic diseases from bench to bedside. Pharmaceuticals, 16: 541.

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