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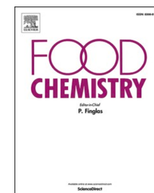

Effect of household cooking on the retention of vitamin D_2 and 25-hydroxyvitamin D_2 in pulse UV-irradiated, air-dried button mushrooms (*Agaricus bisporus*)

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ABSTRACT

Vitamin D deficiency has widespread global prevalence. Fresh mushrooms exposed to ultraviolet (UV) radiation generate vitamin D_2 which remains after drying. It is not clear if vitamin D_2 is retained after rehydration and cooking of dried mushrooms. The aim of this study was to determine the true retention of both vitamin D_2 and 25-hydroxyvitamin D_2 (25(OH)D_2) after cooking UV-irradiated, air-dried, then rehydrated button mushrooms (*Agaricus bisporus*). Mushrooms were exposed to pulsed UV radiation, then air-dried in a convection oven, followed by rehydration in warm water. Samples were cooked in three different ways: frying (5 min), baking (10 min, 200 °C) and boiling (20 min, 90 °C). Compared to rehydrated, uncooked controls, there was a high retention of D vitamers (≥95%) after cooking. Frying and baking resulted in significantly higher vitamin D_2 retention compared to boiling ($p < 0.0001$). UV-irradiated, dried mushrooms are a valuable source of vitamin D_2 after rehydration and cooking.

1. Introduction

There is a high global prevalence of vitamin D deficiency (Amrein et al., 2020; Cashman, 2022) and low vitamin D intakes have been widely reported (Ahmed, Ng, & L'Abbe, 2021; Dunlop et al., 2023; Herrick et al., 2019). The few foods that are a good source of vitamin D are mainly limited to foods of animal origin, such as fish, meat and egg yolk. Some countries permit the vitamin D fortification of foods such as milk, milk substitutes and breakfast cereals.

Mushrooms are usually treated as a vegetable in culinary practise; however, they are not a plant and reside in their own biological kingdom, namely fungi. Unlike plant foods, edible fungi are rich in ergosterol, also known as pro-vitamin D_2. It is well established that when mushrooms are exposed to a source of ultraviolet (UV) radiation, such as solar radiation or a UV lamp, their ergosterol is converted to pre-vitamin D_2, which is then thermally isomerised to vitamin D_2 in high concentrations in a temperature-dependent process (Cardwell, Bornman, James, & Black, 2018; Jasinghe, Perera, & Sablini, 2007; Keegan, Lu,

Abbreviations: 25(OH)D_2, 25-hydroxyvitamin D_2; DW, dry weight; FW, fresh weight; LOQ, limit of quantitation; LC-QQQ, Liquid chromatography with triple quadrupole tandem mass spectrometry; UV, ultraviolet.

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Bogusz, Williams, & Holick, 2013).

As mushrooms are commonly consumed worldwide, they are a potential non-animal source of vitamin D. There is usually no vitamin D_2 in commercially cultivated mushrooms as they receive only (non-UV) fluorescent light during picking and packing. However, a 100 g serve of fresh UV-exposed mushrooms can produce 20–160 μg of vitamin D_2 depending on duration and intensity of UV radiation (Kalaras, Beelman, & Elias, 2012; Koyyalamudi, Jeong, Pang, Teal, & Biggs, 2011; Kristensen, Rosenqvist, & Jakobsen, 2012; Urbain & Jakobsen, 2015). Therefore, this popular vegetarian and vegan food has the potential to counteract vitamin D deficiency and help people meet the recommended daily dietary intake of 5–20 μg of vitamin D, depending on regional recommendations (EFSA, 2017; Institute of Medicine, 2011; National Health and Medical Research Council, 2006; Nordic Council of Ministers, 2014).

Air-drying mushrooms is a simple method used to inhibit spoilage from microorganisms, permit storage at room temperature, and extend the shelf life, which are important attributes for populations with little or no access to refrigeration. Dried mushrooms are commonly used in Asian cuisine, yet they have not been well studied as a source of vitamin D. A survey of 35 species of dried mushrooms available in China revealed that all samples contained vitamin D_2 (average 16.9 μg/g dried mushroom; range 7.7–25.0 μg/g), most likely generated by sun exposure during the drying process or during growth of the wild varieties (Huang, Cai, & Xu, 2016).

The impact of cooking on the retention of vitamin D_2 has been explored in fresh UV-exposed mushrooms (Ložnjak & Jakobsen, 2018; Malik, Jan, Haq, Kaur, & Panda, 2022; Mattila, Ronkainen, Lehikoinen, & Piironen, 1999; Salemi et al., 2021). To our knowledge, there are no studies of vitamin D_2 retention in dried mushrooms that were subsequently cooked. Hence, the aim of this study was to investigate the effect of common household cooking methods on the retention of both vitamin D_2 and 25-hydroxyvitamin D_2 (25(OH)D_2) in UV-irradiated, air-dried, common button mushrooms (*Agaricus bisporus*), one of the most highly consumed mushroom species in the world (Royse, Baars, & Tan, 2017).

2. Materials and methods

2.1. Mushroom samples

Forty-one 200 g samples of fresh button mushrooms, 4.5–5.5 cm in diameter, were collected directly from a farm (Costa Mushrooms) in Perth, Western Australia, and transported by road to Curtin University in light-sealed, cooled, insulated boxes (travel time 30 min). The mushrooms were harvested as for market, comprising cap, stipe and with the gills still skirted. The mean weight of each mushroom was 24.2 g (standard deviation (SD) 1.0, range 23.0–25.7 g). Samples were refrigerated at 4 °C and prepared within 48 h of collection. Any residual compost was brushed from the mushrooms, which were kept at room temperature (23 °C) for 2 h prior to further processing.

The fresh mushroom samples were irradiated, followed by air-drying, then randomly allocated to one of five groups for the following processes:

1) Rehydrated, not cooked (control);
2) Rehydrated, dry-fried 5 min;
3) Rehydrated, baked 10 min;
4) Rehydrated, boiled 20 min in deionised water at pH 5.5;
5) Rehydrated, boiled 20 min in deionised water at pH 3.5.

In addition, one random, non-irradiated fresh sample (1 × 200 g) was analysed for D vitamers to confirm there had been no exposure to UV radiation at the farm or during transportation.

2.2. Preparation of rehydrated control samples

Each sample (8 × 200 g) was UV-irradiated under a pulsed xenon lamp (Wek-tec XeMaticA-2L, Gottmadingen, Germany; emitting at 260–800 nm) with the skirted gills facing the lamp. The average dose was 200 mJ/cm^2, with an average peak of 17.5 W/cm^2, based on five pulses at 7 V as measured with a radiometer (ILT800 CureRight, International Light Technologies, Peabody, MA, USA; bandwidth measured 215–350 nm). The distance from the surface of the mushrooms to the lamp was 10 cm. Each mushroom was then placed in a single layer on shelves and air-dried in a convection oven for 18 h at 60 °C (Contherm Thermotec 2000, Contherm Scientific Ltd, Lower Hutt, New Zealand).

The samples were rehydrated in deionised water for 2 h in a covered stainless-steel saucepan. The initial water temperature for that and all subsequent rehydrated samples was 50 °C, reducing to 36 °C after 60 min, and 28 °C at 120 min (Micron Q1090 multimeter, Micron Technology, Boise, ID, USA). The internal mushroom temperature during rehydration was the same as the water temperature. Excess water was removed via sieve and paper towelette before the samples were weighed, sliced and placed in a freezer at −20 °C overnight then lyophilised (Christ Alpha 1–2 LD plus, Osterode am Harz, Germany) at −30 °C and 37 Pa for 48 h, crushed with a mortar and pestle, then blended to a fine powder.

2.3. Preparation of dry-fried mushroom samples

Each 200 g sample (n = 8) was irradiated and air-dried as per Section 2.2, then vacuum-sealed and stored at 4 °C for four days, before being rehydrated and excess water removed, in the same manner as per Section 2.2, prior to frying. A stainless-steel frying pan was pre-heated for 10 min on a gas stove. Within 60 min of rehydration, each 200 g sample was dry-fried, independently of each other, for 5 min. The internal mushroom temperature was measured randomly (n = 23) via the stipe directly after completion of frying with a multimeter probe (mean internal temperature 53 °C; SD 5; range 43–63 °C; median 53 °C). Each sample was then weighed, sliced and placed in a freezer at −20 °C overnight then lyophilised as described in Section 2.2.

2.4. Preparation of baked mushroom samples

Each 200 g sample (n = 8) was irradiated, air-dried, vacuum-sealed and stored at 4 °C for three days until rehydration and preparation for cooking as in Section 2.2. Samples were baked for 10 min in a pre-heated fan-forced LG oven (Model LF66105SS) at 200 °C (Avanti Tempwiz oven thermometer, Avanti Homewares, Sydney, Australia). The mean internal mushroom temperature was randomly measured (n = 12) directly after 10 min of baking was 56 °C (mean 56 °C; SD 8; range 43–65 °C; median 57 °C). After baking, the samples were weighed, sliced and placed in a freezer at −20 °C, then lyophilised as per Section 2.2.

2.5. Preparation of boiled mushroom samples

Each 200 g sample (n = 16) was irradiated, air-dried, vacuum-sealed and stored at 4 °C for four days until rehydration for 2 h in deionised water as in Section 2.2. Samples were boiled for 20 min in 500 mL deionised water at 90 °C in a stainless-steel saucepan within an hour of rehydration. The water temperature was checked every 5 min to ensure the temperature remained constant. The samples were boiled separately at either of two pH levels. Samples (8 × 200 g) were boiled in deionised water that had a native pH of 5.3–5.6 (mean 5.5), while another 8 × 200 g samples were boiled in deionised water with a pH of 3.5 (TPS AQUA-pH meter, Brendale, Queensland, Australia), adjusted by the addition of fresh lemon juice. After 20 min boiling, the internal temperature was measured randomly in 14 individual mushroom samples (mean 82 °C, SD 4; range 74–88 °C; median 82 °C). The samples were then drained with a sieve and dried with a paper towelette, weighed, sliced and

Table 1
Mushroom sample weight (mean ± SD) after each treatment process.

Treatment	Fresh weight (g)	Air-dried weight (g)	% weight loss[1]	Rehydrated weight (g)	RR	Cooked weight (g)	% weight loss[2]	Freeze-dried weight (g)	% weight loss[3]
Fresh[4]	199.2	N/A	N/A	N/A	N/A	N/A	N/A	19.2	90.4
Irradiated, dried, rehydrated (control)[5]	209.1 ± 5.1	25.4 ± 4.0	88 ± 2	57.0 ± 4.9	2.2	N/A	N/A	12.0 ± 0.9	94.2 ± 0.4[b]
Irradiated, dried, rehydrated, dry-fried[5]	207.0 ± 5.3	23.9 ± 2.4	89 ± 1	47.7 ± 4.8	2.0	35.3 ± 1.5	26.0 ± 3.1	10.9 ± 0.3	94.7 ± 0.2[a,b]
Irradiated, dried, rehydrated, baked, oven[5]	206.9 ± 5.8	22.3 ± 1.9	89 ± 1	48.6 ± 2.7	2.2	31.9 ± 2.3	34.4 ± 3.8	11.3 ± 1.4	94.5 ± 0.5[a,b]
Irradiated, dried, rehydrated, dried, boiled, pH 5.5[5]	206.8 ± 5.9	23.4 ± 1.6	89 ± 1	52.8 ± 3.3	2.3	51.0 ± 2.9	3.4 ± 5.1	9.6 ± 0.7	95.4 ± 0.3[a,b]
Irradiated, dried, rehydrated, dried, boiled, pH 3.5[5]	205.6 ± 4.2	21.9 ± 1.7	89 ± 1	51.5 ± 3.7	2.4	49.9 ± 3.2	3.1 ± 5.5	8.9 ± 0.5	95.7 ± 0.3[a,b]

N/A, not applicable; SD, standard deviation; RR, rehydration ratio.
[1] sample weight loss after air-drying compared to fresh weight.
[2] sample weight loss after cooking compared to rehydrated weight.
[3] sample weight loss after freeze-drying compared to fresh weight.
[4] 1 × 200 g independent sample.
[5] 8 × 200 g independent samples.
[a] Statistically significant difference ($p < 0.05$) between the boiled samples and the dry-fried or baked samples.
[b] Statistically significant difference ($p < 0.05$) between the control samples and the cooked samples.

placed in a freezer at −20 °C, then lyophilised as per Section 2.2.

2.6. Dry weight (DW) determination

Prior to analysis, all samples were freeze-dried as per Section 2.2 until there was no further weight loss (48 h). The samples were weighed using an electronic compact balance (EK-4100i, A&D Technology, Ann Arbor, MI, USA).

2.7. Rehydration ratio

As described in Section 2.2, the dried mushrooms were rehydrated in 50 °C deionised water for 2 h. They were weighed directly before rehydration, and after 60 and 120 min of rehydration, with the rehydration ratio (RR) determined by the equation:

$$RR = \text{Rehydrated weight}(g)/\text{Air} - \text{dried weight}(g)$$

2.8. Weight loss and true retention

The samples were weighed initially in their fresh state, then after each step of air-drying, rehydrating, each cooking procedure, and after freeze-drying (Table 1). To determine the percentage weight loss attributed to cooking, compared to the rehydrated weight, we used Eq. (1) below. We used Eq. (2) to determine the true retention of D vitamers, which we adapted from Murphy and colleagues (Murphy, Criner, & Gray, 1975).

DW, dry weight; FW, fresh weight.

2.9. Transport of samples for analysis

Lyophilised samples were stored at −20 °C for two weeks before being sent for analysis at the National Measurement Institute (NMI) in Melbourne, Victoria, Australia. Sealed, freeze-dried samples were placed in an insulated, light-sealed container with frozen cool bricks for overnight transport to the NMI. On arrival, samples were kept frozen at −20 °C before measurement of vitamin D_2 and $25(OH)D_2$ within 8 weeks.

2.10. Analysis of D vitamers

Vitamin D_2 and $25(OH)D_2$ were measured using a validated in-house method at NMI (ISO17025 (International Organization for Standardization, 2017)) approved by the National Association of Testing Authorities, Australia, and previously described (Cardwell, Bornman, James, Daly, Strobel, et al., 2023). It was developed from published methodologies (Jäpelt, Silvestro, Smedsgaard, Jensen, & Jakobsen, 2013; Jones & Makin, 2000; Strobel, Buddhadasa, Adorno, Stockham, & Greenfield, 2013). The entire analysis was conducted in a laboratory protected from UV radiation.

Freeze-dried mushroom powder (500 mg) was added to a 50 mL screw top Falcon® plastic centrifuge tube before the addition of 1000 mg of sodium ascorbate (ChemSupply, Adelaide, South Australia) and 10 mL Milli-Q® water (Millipore Australia, Sydney, New South Wales). The resultant mixture was vortexed before the addition of the internal standard of vitamin D_2-$^2H_3$ and $25(OH)D_2$-$^2H_3$ (IsoSciences, Ambler, PA,

$$\%\ \text{weight loss} = \left(\frac{\textit{weight of rehydrated sample before cooking}\ (g) - \textit{weight of sample after cooking}\ (g)}{\textit{weight of rehydrated sample before cooking}\ (g)}\right)*100 \quad (1)$$

$$\%\ \textit{True retention} = \left(\frac{\textit{Cooked sample}:\ [(D\ vitamer\ \mu g/g\ DW\ x\ total\ DW(g)) \div FW\ (g)]}{\textit{Control sample}:\ [D\ vitamer\ \mu g/g\ DW\ x\ total\ DW\ (g) \div FW\ (g)]}\right)*100 \quad (2)$$

USA; purity ≥98%). Ethanol (30 mL; ChemSupply, Adelaide, South Australia) and Milli-Q® water were added to a total volume of 50 mL. The tubes were then capped and hand-shaken, followed by the addition of 2.0 g potassium hydroxide (ChemSupply, Adelaide, South Australia). The remaining headspace of the Falcon® tube was flushed with nitrogen gas to minimise potential oxidation during saponification. The tube was immediately capped and placed laterally in a shaker bath for saponification at 25 °C for 18 h.

The tubes were centrifuged for 90 s at 1887 *g* (~3750 rpm, radius 12 cm: Pro-Analytical, Centurion Scientific Ltd, Chichester, United Kingdom). A 10 mL aliquot of the supernatant was pipetted onto diatomaceous earth extraction Chem Elut 10 mL unbuffered cartridges (Agilent Technologies, Santa Clara, CA, USA). After 15 min, 60 mL of petroleum ether (boiling point 40–60 °C; ChemSupply, Adelaide, South Australia) were added to the extraction cartridges and the resultant eluent was evaporated with nitrogen gas in a Dionex SE500 Gas Evaporative System (Thermo Scientific, Waltham, MA, USA).

One mL *n*-heptane (Merck KGaA, Darmstadt, Germany) was added to each sample and vortexed. The final volume was transferred to 1.8 mL glass liquid chromatography (LC) vials (Waters Corporation, Milford, MA, USA) and evaporated with nitrogen gas before adding 800 µL of 4-phenyl-1, 2, 4-triazoline-3, 5-dione (PTAD; Sigma-Aldrich, St. Louis, MO, USA) at 2 µg/µL. The samples were allowed to form vitamin D-PTAD derivatives for 10 min, before 200 µL of milli-Q water were added to stop the derivatisation. Of the final volume, 400 µL were pipetted into LC vials. The injection volume of 10 µL was analysed by LC-QQQ (Agilent Technologies, 1290 Infinity Series LC System/6460 Triple Quad LC-MS fitted with a Jet Stream ESI source in positive ion mode). To ensure that only one vitamer-PTAD adduct was formed, 5 mM of methylamine was added to the mobile phase. The LC chromatographic column was a Supelco Ascentis Express C18, 15 cm × 3 mm, 2.7 µm (Cat #53816-U, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). Each sample was analysed in duplicate and the average of the two results calculated. The limit of quantitation (LOQ) was 0.001 µg/g DW for both vitamin D_2 and 25(OH)D_2.

2.11. Statistical analysis

Wilcoxin rank sum tests were used to test the differences in freeze-dried weight between the control sample and the combined samples of each of the different cooking methods, and the differences in vitamin D_2 concentrations between the control sample and the combined samples of the different cooking methods. For the data used in the calculation of the retention of D vitamers, a natural log transform was used, and the results were back-transformed for reporting. The mean fresh weight of the control mushrooms was used as the denominator in the retention formula (see Section 2.8, equation (2). A one-way analysis of variance (ANOVA) with Tukey's pairwise comparisons was used to test statistically significant differences in the retention of vitamin D_2 and 25(OH)D_2 between cooking methods. ANOVA was also used to test the differences in freeze-dried weight between the control mushrooms and the cooked samples. Data were analysed using Stata (Version 17.0, StataCorp, College Station, TX, USA).

3. Results and discussion

3.1. D vitamers in fresh mushrooms

The concentration of vitamin D_2 and 25(OH)D_2 in fresh mushrooms on arrival at the laboratory was below the Level of Quantitation (LOQ), confirming that there was no exposure to UV radiation at the farm or during transportation.

3.2. Air drying of fresh mushrooms

A temperature of 60 °C was chosen as it is the common air-drying temperature used in commercial settings (Argyropoulos, Heindl, & Müller, 2011; Giri & Prasad, 2009). Samples were irradiated before air-drying as that has been found to be more efficient at generating D vitamers than air-drying prior to irradiation (Cardwell, Bornman, James, Daly, Strobel, et al., 2023). The air-drying method was similar to that used in four studies of dried mushroom: three with sliced white button mushrooms and one with whole shiitake mushrooms (Doymaz, 2014; García-Segovia, Andrés-Bello, & Martínez-Monzó, 2011; Giri & Prasad, 2007; Giri & Prasad, 2009).

3.3. Rehydration of dried mushrooms

During the rehydration process the water temperature dropped from an initial 50 °C to a mean of 36 °C after 1 h, and 28 °C after 2 h. The water temperature was allowed to decline over time in order to replicate preparation in a domestic kitchen where dried mushrooms are left to soak in warm water for one or two hours before cooking. The samples approximately doubled their weight after 1 h of rehydrating in warm water and were 2.2 times their weight after 2 h. Between 90 and 94% of the total rehydration over 2 h was achieved in the first hour. The swift initial water uptake was possibly due to the rapid filling of cavities between cells as the absorption rate begins to decline once the hyphae and intercellular spaces fill with water (García-Segovia et al., 2011).

3.4. Weight differences in freeze-dried samples

Compared with the control, the cooked samples had a higher loss of solid matter after freeze-drying ($p < 0.05$). Boiling the rehydrated mushrooms resulted in a statistically significantly higher loss of solid matter, and hence a lower freeze-dried weight, compared to any of the other cooking methods ($p < 0.0001$ in all comparisons). Compared to fresh mushrooms, the process of rehydrating and cooking may have caused a loss of cellular matter, possibly via dissolving and leaching of dried mushroom particles into the surrounding water.

3.5. D vitamer retention in cooked, rehydrated, UV-irradiated, air-dried mushrooms

The true retention of vitamin D_2 was 109% for dry-fried mushrooms, 108% for baked, 100% for mushrooms boiled at pH 5.5, and 101% for those boiled at pH 3.5. The true retention was statistically significantly lower for vitamin D_2 ($p < 0.0001$) when mushrooms were boiled (at either pH 5.5 or 3.5) compared to mushrooms that were dry-fried or baked. The difference in retention rate of vitamin D_2 between mushrooms that were baked or dry-fried was not statistically significant. There was no significant difference in the retention rate of D_2 between boiling the samples in water of either pH 3.5 or pH 5.5.

The true retention of 25(OH)D_2 was 100% for dry-fried mushrooms, 97% for baked, 95% when boiled at pH 5.5, and 96% for those boiled at pH 3.5. Comparisons between the retention rates for 25(OH)D_2 were not statistically significantly different.

It may not be valid to compare the retention of D vitamers in the current study of cooked, rehydrated dried mushrooms to studies of cooked fresh mushrooms. The true retention of D vitamers in cooked dried mushrooms may be different to that of fresh mushrooms because drying affects the cellular structure. However, it appears that the true retention of vitamin D_2 in this study of cooked dried mushrooms is generally higher than that found in fresh cooked button mushrooms, which has been reported as 94% (roasted) and 92% (boiled) (Salemi et al., 2021), 88% (boiled), 75% (roasted) and 67% (fried in oil) (Malik et al., 2022), 81–88% (fried, no oil), 62–67% (baked), and 62–80% (boiled) (Ložnjak & Jakobsen, 2018).

3.5.1. Boiling

There was nearly complete retention of D vitamers in boiled, rehydrated dried mushrooms at the two different pH values compared to the

Table 2
True retention of D vitamers in dried, rehydrated and cooked mushrooms (mean ± SD).

	Cook temp (°C)	Time (min)	Mushroom internal temp (°C)	Vitamin D_2 µg/g DW	True retention % vitamin D_2	25(OH)D_2 µg/g DW	True retention % 25(OH)D_2
Fresh[2]	N/A	N/A	N/A	<LOQ[1]	N/A	<LOQ[1]	N/A
Irradiated, dried, rehydrated (control)[3]	N/A	N/A	N/A	14.8 ± 3.0[a]	N/A	0.25 ± 0.03	N/A
Irradiated, dried, rehydrated, dry-fried[3]	n.m.	5	53 ± 5.0	17.9 ± 1.9[a]	109 ± 3[b]	0.27 ± 0.03	101 ± 3[c]
Irradiated, dried, rehydrated, baked, oven[3]	200 °C	10	56 ± 7.5	17.7 ± 3.1[a]	108 ± 4[b]	0.23 ± 0.05	97 ± 4
Irradiated, rehydrated, dried, boiled, pH 5.5[3]	90 °C	20	82 ± 3.9	16.6 ± 1.0[a]	100 ± 2[b]	0.25 ± 0.04	95 ± 4[c]
Irradiated, rehydrated, dried, boiled, pH 3.5[3]	90 °C	20	82 ± 3.9	17.9 ± 1.6[a]	101 ± 2[b]	0.28 ± 0.07	96 ± 6

25(OH)D_2, 25-hydroxyvitamin D_2; DW, dry weight; LOQ; level of quantitation; N/A, not applicable; n.m., not measured; SD, standard deviation.
[1] LOQ < 0.001 µg/g.
[2] 1 × 200 g independent sample.
[3] 8 × 200 g independent samples.
[a] $p < 0.05$ difference between the control samples and the cooked samples.
[b] $p < 0.0001$ difference between the boiled samples and the dry-fried and baked samples.
[c] $p = 0.05$ between the dry-fried sample and the sample boiled at pH 5.5.

control samples (Table 2). There was no statistically significant difference in D vitamer retention between rehydrated dried mushrooms boiled at either pH 3.5 or 5.5. There was an expectation that there would be a difference in D vitamer retention at the two different pH values based on the results of another study (Ložnjak & Jakobsen, 2018). In that study, the retention of vitamin D_2 was 80% when whole fresh mushrooms were boiled for 20 min in water with added lemon juice to a pH of 3.5, compared to 62% when boiled in neutral-pH water. The authors of that study suggested that the lemon juice preserved the vitamin D during cooking, possibly due to the antioxidant properties of ascorbic acid (Hajimahmoodi, Aliabadipoor, Moghaddam, Sadeghi, Oveisi, & Jannat, 2012). Two further studies of fresh UV-irradiated button mushrooms that were boiled (the water pH was not documented) also found a high vitamin D_2 retention of 92% (Salemi et al., 2021) and 88% (Malik et al., 2022).

3.5.2. Frying

Dry-fried mushrooms retained all their D vitamers compared to the control samples (Table 2). This was a higher level of retention than found in two studies of fresh mushrooms that were also fried without added fat for 5 min. In those studies, the true retention was 81% in whole fried button mushrooms (Ložnjak & Jakobsen, 2018), and 80–82% and 97–100% in fried wild mushrooms (*Cantharellus cibarius* and *Cantharellus tubaeformis*, respectively) (Mattila et al., 1999). In comparison, another study showed that frying fresh sliced UV-irradiated button mushrooms in oil resulted in a 67% retention of vitamin D_2, possibly due to the fat-soluble vitamin D_2 leaching into the oil (Malik et al., 2022).

3.5.3. Baking

The baked mushrooms retained 108% of their vitamin D_2 and 97% of 25(OH)D_2, which is higher than in other studies of baked or roasted fresh UV-irradiated mushrooms. In a similar study, but using whole fresh button mushrooms, there was 67% retention of vitamin D_2 in mushrooms baked under the same conditions as the current study (Ložnjak & Jakobsen, 2018). Two other studies of vitamin D_2 retention in roasted fresh UV-irradiated button mushrooms found 75% retention in sliced fresh mushrooms (Malik et al., 2022), and a 94% retention in whole fresh mushrooms (Salemi et al., 2021).

There is a consistency in that all studies of cooked fresh and dried UV-irradiated mushrooms have shown a high retention of vitamin D_2 such that they offer nutritionally relevant amounts of D vitamers to the consumer. Although the mushrooms were cooked within four days of drying, it is also worth noting that UV-irradiated dried mushrooms have at least 50% retention of D-vitamers after 12 months storage, suggesting a long-term potential for dried mushrooms as a D-vitamer source (Cardwell, Bornman, James, Daly, Dunlop, et al., 2023; Sławińska et al., 2016). Dried wild mushrooms and sun-dried commercial mushrooms may also have the potential to be a source of D vitamers when cooked (Huang et al., 2016; Rangel-Castro, Staffas, & Danell, 2002). Commercial packs of dried mushrooms usually state a serving size as 10–30 g. Using an example serving size of 20 g, the UV-irradiated, air-dried then rehydrated control samples would provide approximately 140 µg D_2, which is higher than the recommended daily requirements for vitamin D. The UV dose could be modified to generate amounts of vitamin D to suit the local requirement for dietary vitamin D, or the dried mushrooms could be ground into a powder to provide a supplemental source of vitamin D for use in dishes such as soups and stews, or as an ingredient in manufactured food products (Heo, Kim, Park, & Lee, 2020). The European Union has approved the sale of fresh UV-exposed mushrooms provided that the tolerable upper intake limits of vitamin D set by the European Food Safety Authority (EFSA) are not exceeded (European Commission, 2018). In 2019, EFSA also approved the use of UV-exposed mushroom powder as a novel food ingredient (Turck et al., 2020).

4. Strengths and limitations

The mushrooms were collected directly from the farm and analysis of the fresh mushrooms showed that the D vitamers were below the limit of quantitation, confirming they had not been inadvertently exposed to sunlight or other source of UV radiation prior to treatment. The irradiation dose was measured between each sample treatment to provide an average dose over the entire exposure process and to ensure that irradiation was consistent over time. Although the current study used common drying and rehydration times and temperatures, the D vitamer retention may vary depending on drying and rehydrating conditions. The rehydration ratio may have been higher if rehydration was maintained at a constant water temperature and continued until there was no further weight gain. However, the rehydration method was designed to mimic home rehydration prior to cooking. Only one dose level of UV radiation was used in this study since it has been previously reported that the dose was sufficient to provide a nutritionally useful concentration of D vitamers (Cardwell, Bornman, James, Daly, Dunlop, et al., 2023); different doses will generate different concentrations of D vitamers.

5. Conclusion

In conclusion, UV-irradiated, air-dried mushrooms that were rehydrated in warm water and then cooked, retained a high proportion of their D vitamer content. Dried mushrooms that have been biofortified with D vitamers via UV radiation can be a very useful, convenient and affordable source of vitamin D_2 even after cooking using common household methods. They are likely to benefit populations without access to refrigeration, people avoiding animal derived products for religious, cultural or ethical reasons, and those at a high risk to vitamin D deficiency.

CRediT authorship contribution statement

Glenn Cardwell: Conceptualization, Investigation, Methodology, Data curation, Writing – original draft. **Janet F. Bornman:** Methodology, Writing – review & editing. **Anthony P. James:** Methodology, Writing – review & editing. **Alison Daly:** Writing – review & editing. **Georgios Dabos:** Methodology. **Paul Adorno:** Resources. **Jette Jakobsen:** Methodology, Writing – review & editing. **Eleanor Dunlop:** Writing – review & editing. **Lucinda J. Black:** Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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