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Review

Safety assessment of the post-harvest treatment of button mushrooms (*Agaricus bisporus*) using ultraviolet light

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ABSTRACT

Wild mushrooms are an excellent source of vitamin D. The presence of vitamin D in mushrooms is attributed to sunlight exposure, which catalyzes the conversion of fungal ergosterol to vitamin D_2 *via* a series of photochemical/thermal reactions. Mushroom growers now incorporate UV light treatments during processing to produce mushrooms with levels of vitamin D that compare to those in wild mushrooms. Presented herein is a comprehensive review of information relevant to the safety of introducing vitamin D mushrooms, produced using UV light technologies, to the food supply. Historical reference to the use of UV light for production of vitamin D is discussed, and studies evaluating the nutritional value and safety of vitamin D mushrooms are reviewed. Traditional safety evaluation practices for food additives are not applicable to whole foods; therefore, the application of substantial equivalence and history-of-safe-use is presented. It was demonstrated that vitamin D in mushrooms, produced using UV light technologies, are equivalent to vitamin D in mushrooms exposed to sunlight, and that UV light has a long-history of safe use for production of vitamin D in food. Vitamin D mushrooms produced using UV light technologies were therefore considered safe and suitable for introduction to the marketplace.

Contents

Abbreviations: FDA, U.S. Food and Drug Administration; FNB, Food and Nutrition Board; GRAS, Generally Recognized as Safe; HOSU, history of safe use; HPLC, high performance liquid chromatography; IOM, Institute of Medicine's; OECD, Organisation for Economic Co-operation and Development; PTH, phenylthiohydantoin amino acid; RDA, Recommended Dietary Allowance; U.S., United States; UL, Upper Level; UV, ultraviolet; WARF, Wisconsin Alumni Research Foundation.

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1. Introduction

Rickets and osteomalacia, the diseases of extreme vitamin D deficiency, are rarely encountered today; however, vitamin D insufficiency remains a problem, exacerbated in populations residing in sub-equatorial latitudes where endogenous production of vitamin D from sunlight is limited during winter months. Foods naturally enriched in vitamin D are limited and strict food fortification policies further limit the availability of vitamin D in the food supply. It is therefore difficult to achieve adequate dietary intakes of vitamin D through the consumption of balanced western diets, and there is a need to encourage the use of vitamin D in foods and/or the increased consumption of underutilized sources of vitamin D (*e.g.*, fish and milk). An unappreciated food source of vitamin D is the mushroom, and all edible mushroom species available on the market contain vitamin D (Phillips et al., 2011). In particular, various species of wild mushrooms are excellent sources of vitamin D, which can in some instances rival concentrations present in oily fish. For example, the concentrations of vitamin D present in wild harvested Chanterelle mushrooms is reported to range between 10.7–29.8 μg/100 g serving [428–1192 IU/serving] (Mattila et al., 1994; Outila et al., 1999; Teichmann et al., 2007). Wild Porcini mushrooms have been shown to contain as much as 58.7 μg/100 g serving (2348 IU/100 g serving), and sun-dried Shitake mushrooms may contain up to 40 μg/100 g serving (1600 IU/100 g serving) (Teichmann et al., 2007; Health Canada, 2012). The presence of large quantities of vitamin D in wild mushrooms is attributed to their growth outdoors, which results in their exposure to sunlight. The ultraviolet radiation present in sunlight catalyzes a unique photochemical reaction whereby the fungal sterol, ergosterol, is converted to vitamin D_2 through a series of photochemical and thermal reactions; this photochemical process is similar to the process by which vitamin D_3 is produced in human skin. To simulate the natural process of vitamin D synthesis that occurs in mushrooms grown in their natural environments, commercial mushroom growers have recently incorporated sources of ultraviolet (UV) light into their production processes. To date, a comprehensive and systematic evaluation of the suitability and safe use of UV light technology for production of vitamin D enriched mushrooms has not been reported. Therefore, the primary objective of this investigation is to present a safety assessment of vitamin D mushrooms produced using UV light technology.

2. Vitamin D background

Vitamin D is a 9, 10 secosteriod, that was first discovered and named by Dr. E.V. McCollum in 1922 as the factor present within cod liver oil that cured rickets in experimental beagle dogs (DeLuca, 2004). Although multiple forms of the vitamin have been identified (vitamins D_2 through D_7), only vitamins D_2 (fungal origin), and D_3 (animal origin) (Fig. 1), are relevant to human nutrition. Vitamin D_3 is synthesized in the epidermis of humans and other animals following exposure of the skin to UVB irradiation from sunlight. Vitamin D_2 is of fungal origin, and is typically obtained from UV irradiation of ergosterol (provitamin D_2) isolated from Baker's yeast (*Saccharomyces cerevisiae*). Vitamin D_2 and D_3 differ only in their side chain structure (Fig. 1). Although some investigators have reported that vitamin D_3 is biologically superior to vitamin D_2 when provided at high doses (Heaney et al., 2011), it is clear that at low doses both forms of the vitamin are nutritionally equivalent (IOM, 2011). Vitamins D_2 and D_3 are biologically inert, and only gain biological activity following a series of hydroxylation steps in the liver and kidneys yielding 1,25-dihydroxyvitamin D the hormone responsible for receptor mediated interactions within vitamin D responsive tissues.

The synthesis of vitamin D_2 by mushrooms (and vitamin D_3 in human skin) occurs *via* the photochemical conversion of provitamin D (*i.e.*, ergosterol in mushrooms, and 7-dehydrocholesterol in

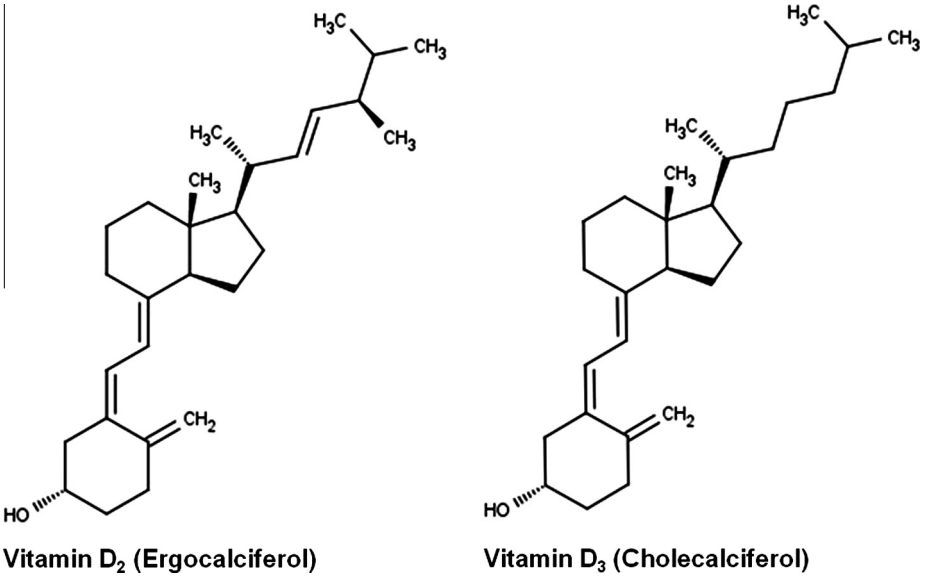

Fig. 1. Comparison of structures of vitamin D_3 and D_2.

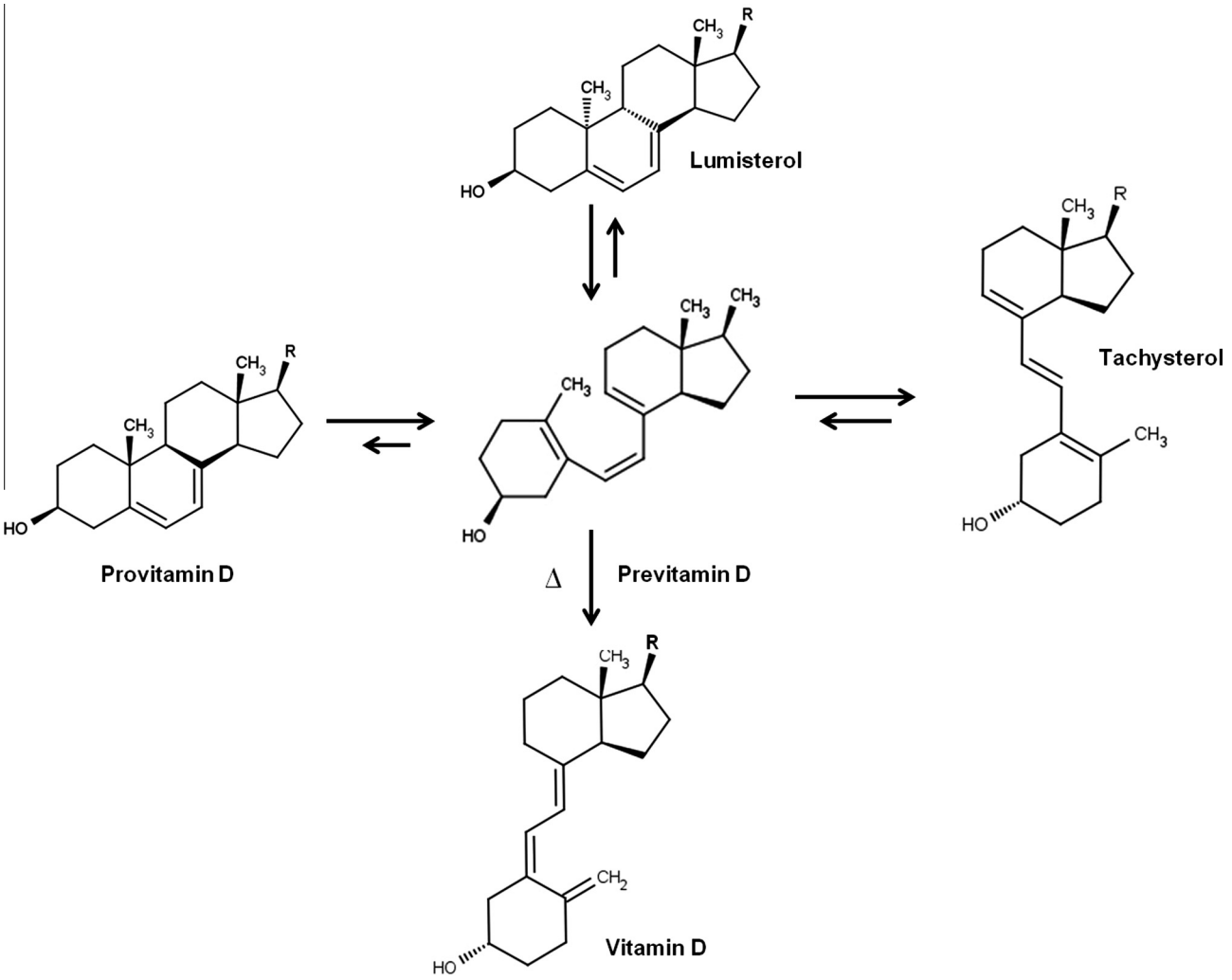

Fig. 2. Photochemical reaction scheme of for conversion of provitamin D to vitamin D and isomers.

human skin) to vitamin D *via* series of photochemical and thermal reactions. These reactions have been fully explored, and were first characterized by the work of Havinga and Velluz (Velluz et al., 1949; Havinga, 1973). Briefly, exposure of provitamin D to UV radiation in the region of 280–315 nm, results in the opening of the B-ring 5,7 diene of provitamin D to form previtamin D. The optimum wavelength for production of vitamin D is 296.5 nm (Havinga et al., 1960). Previtamin D also absorbs UV light in same region resulting in photoisomerization to tachysterol or ring closure to provitamin D and lumisterol (Fig. 2). Previtamin D is then slowly isomerized to vitamin D *via* a thermal reaction. The ratio of photoreaction products at the stationary state are influenced by the irradiation wavelength, with shorter wave-lengths (248–254 nm) producing tachysterol as the major product, and longer wavelengths (>305 nm) favoring the formation of the ring closed isomers provitamin D and lumisterol at the photostationary states (Braun et al., 1991). Prolonged irradiation periods result in the production of the irreversible secondary reaction products toxisterols and suprasterols (Boomsma et al., 1977). During the industrial production of food grade vitamin D, the production of secondary photoreaction products is controlled by termination of the irradiation process at a low conversion of the staring material (ergosterol). The short duration of UV light treatment during commercial processing of vitamin D enhanced mushrooms similarly serves to prevent the formation of secondary products in mushrooms (Kalaras et al., 2012). HPLC analyses of commercial sources of vitamin D mushrooms processed under UV light have not identified detectable peaks suggestive that measurable concentrations of tachysterol or lumisterol are produced in the mushrooms (Phillips et al., 2012).

3. History of safe use

The application of history of safe use (HOSU) in the safety assessment of foods and food ingredients has broad acceptance world-wide (Constable et al., 2007). In the United States, the concept of common use in food has been applied to the safety assessment of Generally Recognized as Safe (GRAS) substances (U.S. FDA, 1997). HOSU forms a fundamental basis by which foods derived from new plant varieties are evaluated (U.S. FDA, 1992). Similar provisions for consideration of HOSU within the safety assessment of foods produced using new/novel technologies are practiced by regulatory bodies internationally (EC, 1997; FAO/WHO, 1996; FSANZ, 2007; Health Canada, 2006). To establish the history of safe use of a food or novel food processing method (*e.g.*, wild mushrooms, UV light treatment of food) documented evidence should exist to show that historical consumption of the food of interest has occurred by humans over a sufficient duration of time, and within a defined population(s), to conclud that the existing history

of exposure has demonstrated a reasonable certainty of no harm to the majority of consumers (ILSI, 2008). Information obtained from the HOSU assessment is deemed relevant to the safety assessment of a "new food" if it can be established that the traditional food with a history of safe consumption (wild sun exposed mushrooms) is substantially equivalent to the food of interest (vitamin D mushrooms produced using UV light technology). Substantial equivalence embodies the concept that if the "new food" can be shown to be compositionally comparable to an existing food that is already considered safe, then the "new food" also would be considered safe (FAO/WHO, 1996). This approach is used by the U.S. Food and Drug Administration (FDA) to assess the safety of new or modified substances derived from new plant varieties, for traditional foods processed using new enzyme preparations, and for foods or food ingredients produced using new technologies or manufacturing methods (U.S. FDA, 1992). Instances where suitable evidence documenting history of safe use are insufficient to establish reasonable certainty of no harm require additional scientific evidence to support safety, which may include hazard characterization of the substance/food through use of validated toxicity models in animals, information detailing pharmacokinetics and metabolism of the substance or food constituent of interest, or the conduct of hypothesis based studies on animals and/or human subjects (*e.g.*, glucose metabolism for individuals with diabetes, allergenicity studies, nutrient bioavailability, or general evaluations of tolerance and safety).

3.1. Vitamin D in mushrooms

Ergosterol (provitamin D_2) was first identified in mushrooms in 1811 by Bracconnot (Bills, 1938), and it has long been known that wild mushrooms are of value to human nutrition: "*...a natural source of supply for vitamin D is opened up by the mushrooms that is of great importance for nutrition. Vitamin D has not been demonstrated in any of the common species of vegetable or fruits, so that mushrooms occupy a unique place and supply a need*" (JAMA, 1931). Unlike plants, mushrooms do not require photosynthesis for growth, and for economic reasons, most common mushroom species are cultivated indoors in dark cool environments without exposure to sources of ultraviolet light. The majority of mushroom species available in the marketplaces of North America and Europe, [*Agaricus bisporus* (white and brown button mushroom strains, which include the Portobello), *Lentinus edodes* (Shiitake), *Boletus edulis* (porcini), and *Pleurotus ostreatus* (oyster mushroom)] contain nutritionally insignificant concentrations of vitamin D. Wild-harvested mushrooms such as the Chanterelle, are available in the marketplace on a seasonal basis and contain nutritionally relevant concentrations of vitamin D (Phillips et al., 2011). The Shiitake mushroom is the second most widely cultivated mushroom in the world (FAO/WHO, 2002). In Japan, commercial cultivation of the Shiitake using traditional natural log inoculation methods grown outdoors remains a common practice. Many growers also have adopted the use of greenhouses to maintain "outdoor" production during the winter months (Royse et al., 1985). Japan remains a world-leader in Shiitake mushroom production. World production of Shiitake mushrooms in 1985 was reported to be 206,700 metric tons, of which 82.8% was accounted for by Japanese growers (Royse et al., 1985). Sixty percent (60%) of all Shiitake mushrooms consumed during this period were de-hydrated using sun drying methods or hot air convection systems (Royse et al., 1985). Thus, the consumption of vitamin D containing sundried mushrooms and mushrooms grown outdoors has a long and widespread history of consumption. A summary of published vitamin D analyses conducted on various wild-harvested, and commercially available UV treated mushrooms is presented in Table 1. The concentrations of vitamin D within other natural food sources also have been included for comparison.

3.2. Current and historical use of UV light for production of vitamin D

Current pharmaceutical and food grade sources of vitamins D_2 and D_3 used for fortification of food and supplement products are typically high purity crystalline forms. For synthetic production of crystalline vitamin D_2, ergosterol is isolated from Baker's yeast *via* solvent extraction and is purified using crystallization and separation techniques. For production of vitamin D_3, 7-dehydrocholesterol, typically obtained from sheep's wool lanolin or *via* chemical synthesis from cholesterol, is used as the starting material. These purified provitamin D isolates are then dissolved in organic solvents and converted to vitamin D using UV light in the range of 250–350 nm. Following removal of the organic solvent a resin containing approximately 70% vitamin D and other photoreaction products (primarily lumisterol and tachysterol) is produced. In the United States, this semi-purified vitamin D resin can be "*...sold as food sources of vitamin D without further purification.*" (U.S. FDA, 2012a); however, in most instances the resins are further purified using chemical esterification and separation techniques. For example, the resin can be further dissolved in an organic solvent and converted to an ester, most commonly 3,5-dinitrobenzoate, which is then separated from other isomer esters and impurities. The vitamin D_2 ester is saponified, and vitamin D_2 is separated and re-crystallized to yield crystalline vitamin D.

Although it is widely recognized that the eradication of rickets was achieved through the fortification of foods with vitamin D in the early 20th century, it is not appreciated that vitamin D fortification in the United States was not first achieved by the direct addition of vitamin D to foods, but by the UV irradiation of foods using patented procedures that were developed by Dr. Harry Steenbock of the University of Wisconsin–Madison. Dr. Steenbock's work lead to the creation of the Wisconsin Alumni Research Foundation (WARF) that was founded on November 14, 1925 to administer Steenbock's vitamin D "fortification" patents (Schneider, 1973; Rajakumar et al., 2007; WARF, 2012). The misbranding and adulteration of food in the early 20th century were unfortunate common practice, and Dr. Steenbock recognized that control of UV irradiation technology through application of patents was necessary to ensure that irradiated vitamin D products introduced to the marketplace were efficacious and safe. The first license for irradiation of food was granted by WARF to the Quaker Oats company in February of 1927 for the manufacture of vitamin D enriched breakfast cereal (WARF, 2012). The most important application of Dr. Steenbock's irradiation technology was for the irradiation of milk, a common staple food to which the direct addition of anything was prohibited under strict food purity laws of the time. Dr. Steenbock's patents expired in 1945, and new regulatory amendments to the 1938 Federal Food Drug and Cosmetic Act eventually led the way to the direct fortification of milk and other food products that remains today.

3.3. Substantial equivalence

Wild grown vitamin D mushrooms have a long history of safe consumption in the food supply. Information demonstrating that vitamin D in mushrooms produced from exposure to the sun are substantially equivalent to vitamin D in mushrooms produced by UV light technology is required to establish the safety of the UV-treated mushrooms. The substantial equivalence process involves a critical evaluation of the compositional profiles of the approved/conventional food or food ingredient with the "new" food or food ingredient (ILSI, 2004). In the case of vitamin D mushrooms, unintended effects of UV light application during mush-

Table 1
Vitamin D content of commercial UV processed mushrooms and comparison to wild mushrooms and other natural food sources of vitamin D.

Food	Vitamin D content μg/100 g serving[a]	Vitamin D content IU/100 g serving[a]	References
Commercial UV processed mushroom			
Portobello (*Agaricus bisporus*)	2.36–20.9	134–836	Phillips et al. (2011)
Portobello (*Agaricus bisporus*)	11.15	446	USDA SR25 (2012)
Wild mushrooms			
Maitake	29.5	1181	USDA SR22 (2009a)
Maitake	0.08–63.2	3.2–2528	Phillips et al. (2011)
Wild Chanterelle (*Cantharellus tubaeformis*)	29.8	1192	Mattila et al. (1994)
Wild Chanterelle (*Cantharellus tubaeformis*)	23.4[b]	936[b]	Outila et al. (1999)
Wild Chanterelle (*Cantharellus cibarius*)	12.8	512	Mattila et al. (1994)
Wild Chanterelle (*Cantharellus cibarius*)	10.7–21	428–840	Teichmann et al. (2007)
Chanterelle (*Cantharellus californicus* and *Cantharellus cibarius*)	2.18–8.41	112–336	Phillips et al. (2011)
Morel (*Morchella esculenta*)	2.18–8.41	112–336	Phillips et al. (2011)
Morel (*Morchella esculenta*)	4.39–6.26	175–250	Phillips et al. (2011)
Wild Porcini (*Boletus edulis*)	58.7	2348	Teichmann et al. (2007)
Sun-dried Shitake (*Lentinula edodes*)	40	1600	Holick (2007)
Sun-dried Shitake (*Lentinula edodes*)	42	1666	Health Canada (2012) (CNF)
Oyster (*Pleurotus ostreatus*)	0.07–2.59	2.8–104	Phillips et al. (2011)
Wild button (*Agaricus bisporus*)	0.7–2.3	28–92	Kristensen et al. (2012)
Sea food			
Filefish	69	2760	JFCD (2004)
Cod Liver Oil	55.4	2217	USDA SR21
Wild Atlantic Salmon	51.5	2061	USDA SR21
Salmon Roe	47	1880	JFCD (2004)
Pink Salmon Raw	24	960	Health Canada, 2012 (CNF)
Wild Salmon	24.7 ± 13.1	988 ± 524	Lu et al. (2007)
Fresh Salmon	1–20	40–800	Ostermeyer and Schmidt (2006)
Smoked Salmon	5–27	200–1080	Ostermeyer and Schmidt (2006)
Canned Kippered Herring	21–50	840–2000	Lunde et al. (1937)
Canned Brisling	25–75	1000–3000	Lunde et al. (1937)
Channel Catfish Wild	26.3	1053	USDA SR21
Atlantic Herring	40.7	1628	USDA SR21
Eastern Oyster	23.5	941	USDA SR21
Chum Salmon	39	1560	JFCD (2004)
Sockeye Salmon	38	1520	JFCD (2004)
Sockeye Salmon	23	920	USDA SR21
Blue fin Tuna	18	720	Health Canada (2012) (CNF)
Canned Tuna	5.8	230	Holick (2007)
Atlantic Halibut	2–50	80–2000	NIFES (2010)
Cod	1.3–6.9	52–276	Ostermeyer and Schmidt (2006)
Pollack	3–10	120–400	Ostermeyer and Schmidt (2006)
Herring	6–15	240–600	Ostermeyer and Schmidt (2006)
Mackerel	1–50	40–2000	Ostermeyer and Schmidt (2006)
Sardine	1.2–135	48–5400	Ostermeyer and Schmidt (2006)
Carp	0.2–25	8–1000	Ostermeyer and Schmidt (2006)
Other			
Tofu	14.5	581	USDA SR21
Egg yolk	0.5	20	Holick (2007)
Sunlight exposure (5–10 min)[c]	75	3000	Holick (2007)

[a] USDA SR21 (2008) estimates are expressed as quantity of vitamin D per 200 cal serving.
[b] Converted to FW from dry weight data.
[c] Erythemal dose of ultraviolet B radiation would be absorbed after an average of 5–10 min of exposure (depending on season, weather, skin type and geographical location) of the arms and legs to direct sunlight.

room processing on macronutrients, micronutrients, and anti-nutrients/naturally occurring toxins, which may not occur in wild mushrooms exposed to sunlight, were evaluated (Simon et al., 2011). Due to environmental and genetic factors that influence the composition of mushrooms, it is not possible to directly compare commercial vitamin D mushrooms grown indoors to mushrooms grown in the wild. Therefore, to avoid confounding by extrinsic factors that are known to affect mushroom composition, mushrooms used for compositional testing were obtained from a single farm, using a single strain of common white button mushroom (*A. bisporus*), which were then processed in the presence or absence of UV light under conditions representative of commercial processing (Fig. 3). A subset of the freshly harvested mushrooms was transferred to a confined location outdoors for direct sunlight exposure to serve as the sunlight comparator. The duration of sunlight exposures were targeted to provide comparable levels of vita-

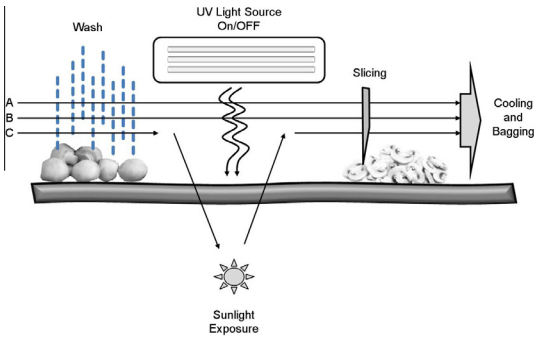

Fig. 3. Schematic overview of typical UV mushroom processing procedure used for production of vitamin D mushrooms.

min D to those produced in the UV light processed mushrooms, and also with consideration of the levels of vitamin D that have been shown to occur naturally in wild mushrooms. Simon and colleagues reported that UVB light exposure at a dose of 1.0 J/cm^2 resulted in the production of 25.2 μg of vitamin D_2 (1008 IU vitamin D) per 84 g fresh weight serving of mushrooms. Comparable concentrations of vitamin D were observed in the mushrooms exposed to sunlight for 2.5 h, *i.e.* 23 μg of vitamin D_2 (918 IU) per 84 g fresh weight serving of mushrooms.

The targeting of specific components for analytical assessment is determined on a case-by-case basis and should consider available information characterizing the known macro- and micronutrients, bioactive substances, allergens, anti-nutrients, and naturally occurring toxins (ILSI, 2004). The Organization for Economic Co-operation and Development (OECD) task force for the Safety of Novel Foods and Feeds has produced a number of science-based consensus documents, which detail the compositional analytes that should be considered for assessment of various food crops. A consensus document on the compositional considerations for new varieties of *A. bisporus* was published by the OECD task force (OECD, 2007). Specific nutrients measured for comparative evaluation of the effects of artificial UV light and sunlight on mushroom composition are presented in Table 2. Based on the results of the compositional testing analyses the authors concluded that the intended effect of increasing the vitamin D content of the mushrooms was achieved and that no unintended effects on mushroom composition were observed as a result of artificial UVB light processing. In contrast, a statistically significant loss of riboflavin (-26%; $p < 0.05$), evidence of folate oxidation and an unexplained 9.5% increase ($p < 0.05$) in ergosterol concentration were observed in the sunlight exposed mushrooms. During analyses of mushroom samples for vitamin D, the presence of an unknown peak eluting with vitamin D_2 was noted in the high performance liquid chromatography (HPLC) chromatogram. A review of the literature for studies evaluating sterol biosynthesis in mushrooms and fungi was conducted to identify sterols containing the light sensitive B-ring 5,7 diene that is expected to be present in provitamin D compounds. A putative provitamin D_4 sterol, 22,23-dihydroergocalciferol, was subsequently identified, and further chemical characterization by Phillips and colleagues confirmed the identity of the unknown compound to be vitamin D_4 (Phillips et al., 2012). As reported by the authors, the concentration of vitamin D_4 produced in UV processed *A. bisporus* mushrooms is low, typically 1/10th of the vitamin D_2 concentrations (Phillips et al., 2012). Higher concentrations of vitamin D_4, in some instances equivalent to vitamin D_2 were noted in select wild mushroom samples. Although it is unknown whether the observed differences in vitamin D_4 concentration between UV exposed mushrooms and sun exposed mushrooms are a reflection of species differences or environmental factors, controlled studies of UV and sunlight exposed mushrooms show that the capacity of UV light processing and sunlight to produce vitamin D_4 is equivalent (Phillips et al., 2011).

Table 2
Nutritional and compositional parameters to be analyzed in mushroom composites.

Nutrients
Proximates
Moisture
Amino acids
Fatty acids
Vitamins
Vitamin D
Vitamin B_6
Vitamin C
Riboflavin (B_2)
Niacin
Pantothenic acid
Folates
5-Methyltetrahydrofolate, 5-formyltetrahydrofolate, tetrahydrofolate, and 10-formyl folate
Other
Ergosterol
Agaritine

4. Animal studies

Traditional techniques of toxicology have limited application in the safety of whole foods like mushrooms because animals studies lack the sensitivity to detect minor changes in the composition of whole foods (Munro et al., 1996a,b; ILSI, 2004). Data generated from toxicological studies in which rodents are fed high dietary concentrations of a whole food or macronutrient may be complicated by adverse effects produced from nutritional imbalances. Many plants contain natural toxins and anti-nutritional factors, and the concentration of these factors in the diet through the use of freeze-dried material (to attain high dietary concentrations for toxicological studies) may result in confounding effects that make interpretation of the toxicological data generated difficult. A similar conclusion was reached by the FDA's Task Group during its review of the application of ionizing radiation to food. The FDA's Task Group concluded that feeding irradiated foods "cannot be expected to provide meaningful answers to toxicity questions regarding such irradiated foods", an opinion that was based on the following three reasons:

"(1) nutritional imbalances created in the test animal fed high levels of irradiated or non-irradiated foods would tend to mask any potential toxicological manifestation: (2) the low concentration of potentially toxic radiolytic products in the irradiated foods would prevent significant exaggeration of the amount of radiolytic product in a test diet; and (3) such toxicological test is currently too insensitive to measure toxicity because the concentrations of unique radiolytic products potentially present in the irradiated foods tested are simply too low." (Pauli and Takeuchi, 1986).

The FDA concluded that toxicological testing of irradiated foods is not needed to support a conclusion that such foods are safe.

Although animal tests may lack the sensitivity to evaluate the nutritional or toxicological impact of minor changes in food composition that may be associated with UV light treatment, animal data can provide corroborative evidence to support safety of the "new food". Animal data are particularly useful for nutritionally enhanced foods (bio-assays) since analytical testing cannot define or predict functional effects of these foods. For example, extraction methods used during chemical analysis may mask the presence of an alternative form of a nutrient (*e.g.*, as a glycoside, or fatty acid

ester) with unknown bioavailability. Matrix interactions also can affect nutrient bioavailability of whole foods.

Following a comprehensive review of the literature, relevant studies evaluating bioavailability and potential toxicity of vitamin D from mushrooms were identified. Koyyalamudi et al. (2009) evaluated the bioavailability of UVC treated *A. bisporus* mushrooms. Mushrooms used in the experiment were processed using UVC light (specific dose not reported) and contained vitamin D at a concentration of 17.6 μg (704 IU) vitamin D_2/g dry weight. Groups of 8 male Sprague-Dawley rats (100–120 g) were administered gavage doses 50, 100 or 200 mg/kg UVC treated mushroom powder suspended in saline per kg body weight per day for a period of three weeks. A group of 8 control animals received non-UVC treated freeze-dried mushroom powder. Plasma levels of 25-hydroxyvitamin D_2 [25(OH)D_2] were measured in all animals at the end of the three week treatment period using HPLC-MS. Relative to animals administered the control mushroom powder, three weeks of vitamin D mushroom administration resulted in a statistically significant ($p < 0.0001$) and dose responsive increases in plasma 25(OH)D_2. The authors concluded that "*vitamin D_2 from irradiated A. bisporus button mushrooms was well absorbed and metabolized when fed to rats.*"

The administration of UVB treated Shiitake mushrooms to Wistar rats was evaluated by Jasinghe and colleagues (2005). Thirty (30) male Wistar rats (54.3 ± 5.12 g) were randomized to one of three groups administered the following dietary treatments: (Group 1) vitamin D deficient diet for one week (baseline, $n = 6$); (Group 2) vitamin D deficient diet for one week followed by daily gavage administration of UVB irradiated Shiitake mushroom powder (28 mg) + vitamin D deficient diet for four weeks ($n = 12$); (Group 3) vitamin D-deficient diet for one week followed by gavage administration of non-irradiated mushroom powder (28 mg) + vitamin D-deficient diet for four weeks ($n = 12$). Mushroom test articles were administered as lyophilized powders suspended in deionized water, and each daily dose contained 1 μg (40 IU) vitamin D_2. Control mushrooms did not contain measurable quantities of vitamin D. Following completion of the treatment period, no statistically significant differences in body weight, dietary intake, or femur length were observed between the animals administered either mushroom preparation. A statistically significant elevation in serum 25(OH)D was observed in the rats administered the UVB treated vitamin D mushrooms (129.42 ± 22.0 *vs.* 6.06 ± 1.09 nmol/L for Group 2 *vs.* group 3 respectively; $p < 0.01$). Serum calcium concentrations were 2.28 ± 0.11 mmol/l and 1.60 ± 0.24 mmol/l ($p < 0.01$; Group 2 vs. Group 3) in the rats consuming the UV treated vitamin D mushrooms (Group 2) and control diet (Group 3) respectively. Femur bone mineral density also was increased in the rodents consuming the UVB exposed vitamin D mushrooms. The authors concluded that "*vitamin D_2 from irradiated edible mushrooms is well absorbed, metabolized to 25-hydroxyvitmain D_2, and possesses an active role in bone mineralization in animals.*" Similar findings have been reported by other investigators demonstrating that vitamin D from mushrooms, processed using UVB light, is bioavailable and has beneficial effects on bone growth in rodents (Jasinghe et al., 2006; Lee et al., 2009).

The effect of vitamin D mushrooms (*A. bisporus*), processed with artificial UV light, on measures of safety, bioavailability, and bone growth was recently evaluated by researchers at the Center for Food Safety and Applied Nutrition of the FDA (Calvo et al., 2012). The study was conducted using 300 weanling female Sprague-Dawley rats administered one of five experimental diets for a period of 10 weeks: (1) synthetic control diet containing 1 IU vitamin D/g diet; (2) vitamin D deficient synthetic diet; (3) synthetic diet supplemented with 5% control mushrooms (not exposed to UV light) (0.12 IU vitamin D/g); (4) synthetic diet containing 2.5% UVB treated containing 15 IU vitamin D_2/g diet; or a synthetic diet containing 5.0% UVB treated mushroom containing 30 IU vitamin D_2/g diet. Diets were based on the AIN-93-G formula with modification of the premix to adjust for vitamin D content across the groups as described above. Vitamin D mushroom were produced using UVB light and contained 15 μg (600 IU) vitamin D_2/g on a dry weight basis. Animals in the control, vitamin D deficient and non-UV treated mushroom groups, were estimated to consume 20, 0 or 2.4 IU vitamin D/rat/day respectively (0.5, 0 or 0.06 μg vitamin D/rat/day). Animals consuming the synthetic diets supplemented with 2.5% or 5% UVB treated mushrooms were estimated to consume 300 or 600 IU vitamin D_2/rat/day respectively (7.5 or 15 μg vitamin D/rat/day). Following 10 weeks of treatment, the animals were euthanized, and measures of vitamin D bioavailability (plasma levels of 25(OH)D and PTH), bone strength and microarchitecture were obtained. Tissue samples from the liver, spleen and kidney were isolated from 6 rats within each group for evaluation of the possible development of secondary hyperparathyroidism or toxicity.

The authors reported that all animals gained weight throughout the treatment period, and no differences in growth curves were observed between the groups. Plasma levels of total 25(OH)D (Dia Sorin RIA) were 32 ± 11, 4 ± 3 and 4 ± 3 ng/ml in the control, vitamin D deficient and non-UV mushroom groups respectively. In the animals consuming synthetic diets supplemented with 2.5% or 5% UVB treated mushrooms, the plasma total 25(OH)D concentrations were 118 ± 28 and 159 ± 29 ng/ml respectively. A corresponding suppression of PTH levels were observed in the animals administered the diets supplemented with 2.5% or 5% UVB treated mushrooms: PTH levels were 35 ± 51 and 28 ± 50 pg/ml in the groups respectively, which compared to 66 ± 91 pg/ml in rats administered diets supplemented with non-UVB treated mushrooms. The consumption of vitamin D mushroom diets tended to have beneficial effects on bone status; however, the authors reported that the effect was modest. Histological evaluation of the livers and kidneys were unremarkable. The authors concluded that vitamin D_2 from UVB treated mushrooms "*is bioavailable, safe, and functional in supporting bone growth and mineralization in a growing rat model without evidence of toxicity*."

4.1. Vitamin D_4

As discussed, the photosensitive sterol, 22,23-dihydroergosterol, which has been identified in several mushroom species, is converted to vitamin D_4 at wavelengths of UV light used for commercial UVB light processing of vitamin D mushrooms. Studies conducted using commercial samples of *A. bisporus* mushroom have reported concentration of this sterol between 42 and 95 μg/100 g dry weight (Phillips et al., 2012). These concentrations were typically 1/10 the levels of ergosterol in the samples (475–938 μg/100 g dry weight) (Phillips et al., 2012). The corresponding concentrations of vitamin D_4 measured in the samples were 1/10th the levels of vitamin D_2 that were produced in the mushrooms (Phillips et al., 2012), which suggests that the photochemical yields of vitamins D_2 and D_4 from their respective provitamin forms are quantitatively similar. Although there is limited information on the nutritional value of vitamin D_4, early studies by DeLuca et al. (1968) conducted using vitamin D deficient rats indicate that the concentrations of vitamin D_4 in UV treated mushrooms are unlikely to be nutritionally relevant (DeLuca et al., 1968). In this study the authors compared the biological activity and metabolism of 22,23-^{3}H-vitamin D_4 (vitamin D_4) with 1,2-^{3}H-vitamin D_3 (vitamin D_3) in weanling male rats (species not reported). Prior to vitamin administration, rats were maintained on vitamin D deficient diets for three to four weeks. These diets were previously shown to result in severe vitamin D deficiency characterized by low serum cal-

cium and poor growth. Rats were administered a single gavage dose of tritiated vitamin D_3 or D_4, and euthanized at intervals between 4 and 48 h. The authors reported that tissue uptake, and localization of ^{3}H-vitamin D_4 radioactivity was qualitatively similar to that observed with ^{3}H-vitmain D_3. The major difference between the kinetics of the two vitamers was that ^{3}H-vitamin D_4 was observed to disappear more rapidly from bone, blood, muscle, and kidney than that of ^{3}H-vitmain D_3. Additional studies evaluating the *in vivo* potency of D_4 relative to D_3 also were conducted in rachitic rat and chick models (DeLuca et al., 1968). In these studies the authors used the "in-line" rat model of vitamin D, a validated method widely used during early research on vitamin D. Briefly, the model involves the dietary induction of rickets in weanling rats through administration of vitamin D deficient diets and exclusion from sunlight until the animals develop defined evidence of rickets characterized by "*palpable enlargement of the writs and a slightly waddling gait usually indicate that rickets has developed to the proper degree.*" (Bills et al., 1931). The animals are then administered graded levels of the vitamin D test preparations for 5 days, and the efficacy of the vitamin D treatment was monitored by a standardized evaluation whereby the "*principal criteria of healing are the development of the line at the zone of provisional calcification and the reappearance of bony trabeculae in the metaphyyseal osteoid.*" (Bills et al., 1931). Using this method DeLuca and colleagues reported that vitamin D_4 was two-thirds as active as vitamin D_3 in rats and one-fifth as active in chicks. Similar studies conducted by other investigators have reported that vitamin D_4 was between one-half to three-quarters as potent as vitamin D_3 (Grab, 1936; McDonald, 1936; Windaus and Trautmann, 1937).

5. Human studies

The bioavailability of vitamin D_2 from UV treated mushrooms (*A. bisporus*) was investigated by Stephensen et al. (2012). The study was conducted using a group of healthy male (n = 14) and female (n = 24) adults 20–59 years of age recruited from the University of California. Subjects participating in the study had high baseline serum 25(OH)D levels which ranged from 60 to 100 nmol/L, concentrations that are consistent with significant sun-exposure by the participants. Participants were randomized to one of four groups: (Group 1) negative control group administered non-UV treated mushrooms plus a placebo capsule; (Group 2) UVB treated mushrooms containing a target value of 10 μg (400 IU) vitamin D_2 per serving plus placebo capsule; (Group 3) UVB treated mushrooms containing a target value of 25 μg (1000 IU) vitamin D_2 per serving plus placebo capsule; (Group 4) non-UV treated mushrooms plus 25 μg (1000 IU) of vitamin D_2 supplement capsules. The study was conducted during the months of June through November and subjects were randomized in four cohorts to ensure that seasonal effects were evenly distributed between the groups. Vitamin D mushrooms used during the study were delivered to the test site as fresh produce (Monterey Mushrooms, Watsonville, CA) under similar conditions that consumers would encounter (UV treatment, packaging, shipping, storage, and cooking were similar to commercial production and home preparation). Test meals were provided daily at the test clinic. Mushrooms prepared in-house were cooked for 60 s at full power in a microwave and then portioned with soybean oil and mixed with the cooked entrée. Mushrooms also were provided with take home meals consisting of lean cuisine entre and soybean oil as needed (weekends and upon request). The concentrations of vitamin D_2 in mushrooms consumed by the participants were obtained on select samples throughout the study, and the mean vitamin D_2 levels in the samples were 0.85 ± 0.95, 0.42 ± 0.30, 8.8 ± 2.6, and 17.1 ± 4.3 μg/serving for Groups 1 through 4 respectively. Serum levels of 25(OH)D_2, and 25(OH)D_3 were measured (UPLC MS/MS) in all subjects at baseline, and on weeks 3 and 6. The authors reported that consumption of UVB-treated mushrooms produced statistically significant increases in serum 25(OH)D_2 concentrations on weeks 3 and 6 relative to that observed in subjects consuming the control non-UV-treated mushroom preparations where vitamin D_2 levels remained low (<6 nmol/l) throughout the study. On week 6, changes in serum 25(OH)D concentrations in Groups 1 through 4 were 1.22 ± 1.65, 13.8 ± 2.42, 12.7 ± 1.22, and 32.8 ± 1.26 nmol/l respectively. The authors concluded that vitamin D_2 from UV-treated mushrooms is absorbed and converted to 25(OH)D_2 with an efficiency similar to that seen for vitamin D_2 from supplements.

In a single blind, placebo controlled, parallel group study by Urbain et al. (2011), the bioavailability of vitamin D_2 from UVB treated button mushrooms was evaluated in healthy adults deficient in vitamin D. Twenty-six healthy male (n = 9) and female (n = 17) Caucasian subjects (aged < 45 years, and BMI of 18.5–26 kg/m^2) were recruited from the employees of the University Medical Center Freiburg (Germany) for participation in the study. All subjects included in the study displayed low (⩽50 nmol/l) serum 25(OH)D levels and normal (2.2-2.7 mmol/l) serum calcium levels. Subjects were not permitted to frequent tanning salons, and were instructed not to consume vitamin D supplements or fish oil capsules, and consumption of fish was limited to no more than once per week. The study was conducted during the months of January through March. Subjects recruited to the study were randomized to one of three treatment groups: (1) vitamin D mushroom group (n = 8), vitamin D supplement group (n = 9), or placebo control group (n = 9). For production of the vitamin D mushrooms, whole button mushrooms (*A. bisporus*) were treated with UVB light at a wavelength of 304 nm for 25 min at ambient temperature (21 °C) (1.5 J/cm^2) resulting in vitamin D concentrations of 491 μg/100 g (19,460 IU/100 g) fresh mushrooms. Subjects randomized to the vitamin D mushroom group consumed an experimental soup containing 28,000 IU of vitamin D_2 once weekly (daily equivalent of 4000 IU) provided by inclusion of vitamin D mushrooms in the soup. A placebo orange juice also was consumed by these individuals. The vitamin D supplement group consumed a standard mushroom soup and were administered 28,000 IU of vitamin D_2 provided by the addition of vitamin D_2 supplement drops to orange juice. Subjects in the placebo group consumed 60 IU of vitamin D_2 from a conventional mushroom soup, and a control orange juice. Test soups and orange juice were consumed on four separate occasions at weekly intervals. Serum 25(OH)D was measured at baseline, and weekly thereafter. Serum calcium and iPTH levels were evaluated at time 0, and at the 1-week post-treatment time-point. Within two eating occasions serum 25(OH)D levels were significantly increased in subjects consuming the vitamin D soups (P = 0.0001 *vs.* placebo). At the 2-week post treatment period, the serum levels of 25(OH)D in the vitamin D mushroom, vitamin D supplement, and placebo control groups were 56.7 ± 7.2, 58.0 ± 11.2 and 28.7 ± 8.7 nmol/L (p < 0.0001 *vs.* control for mushroom and supplement groups). No differences in serum iPTH or calcium were observed between treatment groups at any time-point during the study. At the one-week post-treatment period, serum levels of iPTH were 3.2 ± 1.35, 4.24 ± 1.21 and 3.78 ± 1.26 pmol/l (P = 0.327) for subjects within the vitamin D mushrooms, vitamin D supplement, and placebo groups respectively. Serum calcium levels were reported to be 2.43 ± 0.07, 2.40 ± 0.13 and 2.37 ± 0.09 mmol/l (P = 0.442) for the aforementioned groups respectively. The authors reported that the hypercalcemia (serum calcium >2.7 mmol/l) was the main safety endpoint and that serum calcium remained within the reference range at all time points. The authors also stated that "*No physical symptoms were reported during the study.*" The authors concluded that the "*bioavailability of vita-*

min D_2 from vitamin D_2-enhanced button mushrooms via UV-B irradiation was effective in improving vitamin D status in young healthy adults." The authors also concluded that the capacity of vitamin D_2 from UV-B treated vitamin D mushrooms to increase circulating levels of 25(OH)D was equivalent to that observed in subjects consuming the vitamin D_2 supplemented orange juice.

The bioavailability of vitamin D_2 from the consumption of wild Chanterelle mushrooms (*Cantharellus tubaeformis*) has been reported by Outila et al. (1999). Twenty seven volunteers with serum 25(OH)D concentrations <60 nmol/L (mean = 38.5 nmol/L; range of 15–60 nmol/L) were randomly divided into three groups containing nine subjects per group: Subjects in Group 1 were instructed to consume mushrooms providing 14 μg vitamin D_2/day with lunch, Group 2 consumed 14 μg vitamin D_2/day *via* supplement drops, and the third group received no supplementation. Subjects consumed their respective test articles daily for three weeks. Mushrooms administered to subjects in Group 1 were prepared as a lyophilized homogenate containing 2.34 μg (94 IU) vitamin D/g DW. Mushroom powders were consumed in quantities providing 14 μg (560)/day administered as a broth containing 20 g mushroom per liter of hot water. Bioavailability was evaluated by monitoring changes in serum 25(OH)D using a competitive protein-binding assay at weeks 1.5 and 3 of treatment. The authors reported that individuals randomized to the vitamin D_2 treatment groups displayed time dependent increases in serum 25(OH)D levels relative to levels observed in subjects consuming the control diets. No differences in serum intact parathyroid hormone or urinary calcium were noted among the groups at week 3. Strontium absorption, which is an indicator or predictor of calcium absorption, also was not different between the groups at week 3. However, strontium levels were significantly reduced relative to baseline in the subjects not consuming vitamin D, an effect not observed in the supplemental groups.

Finally, a case report also was identified in the literature in which the diagnosis of vitamin D deficiency in a vegetarian patient was self-treated by the individual through the exclusive consumption of fresh mushrooms exposed to UV light (Ozzard et al., 2008). The vitamin D mushrooms were produced by the individual who purchased a UVB bulb from a local hardware store and exposed 200 g of button mushrooms (*A. bisporus*) daily from a distance of approximately 15 cm. Mushrooms were stir-fried before consumption, and consumed on a daily basis for 3 months. Biochemical values obtained at baseline and after 3 months of mushroom consumption demonstrated normalization of vitamin D status in the individual.

6. Nutritional considerations

6.1. Nutritional requirements and toxicity of vitamin D

Vitamin D is required for the homeostasis of calcium and phosphate, and plays an essential role in bone mineralization by regulating the transcription of a number of genes for calcium transporting proteins, bone matrix proteins, and cell cycle regulatory proteins (FAO/WHO, 2002). In addition to these well established nutritional roles, the identification of extraskeletal vitamin D receptor-mediated actions suggest that vitamin D may have broader nutritional functions (Wang et al., 2012). Vitamin D may influence cardiovascular health, immunity, diseases associated with obesity, autoimmune diseases, neurological diseases, and cancer (Plum and DeLuca, 2010). However, the traditional symptoms of vitamin D deficiency refer only to bone health, and include rickets, osteomalacia, and osteoporosis.

It is well established that the toxic effects of acute and chronic vitamin D overconsumption are attributed to hypercalcemia, an effect mediated by the active metabolite 25(OH)D. Hypercalcemia occurring as a result of vitamin D toxicity produces a number of adverse physiological effects including gastrointestinal discomfort, dehydration, disorientation, fatigue, weakness, headache, polydypsia, polyuria, anorexia, nausea, constipation, weight loss, depression, vague aches, stiffness, soft tissue calcification, nephrocalcinosis, hypertension and anemia. In severe cases, hypercalcemia may lead to renal and heart failure or coma and death (Chesney, 1989).

The first comprehensive assessment of the nutritional requirements, toxicology, metabolism, and safety of vitamin D was conducted by the Institute of Medicine's (IOM) Food and Nutrition Board (FNB) through the work of its Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. This analysis was published as part of the report, "Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride" (IOM, 1997). The dietary reference intakes for calcium and vitamin D were re-evaluated in 2011, and the established RDA, and tolerable Upper Level (UL) intake for all life-stage groups are summarized in Table 3.

The safety assessment of a nutritionally enhanced whole food should consider information characterizing the range, variability, and magnitude of changes in the nutrient of interest. Information on the estimated dietary exposure to the nutrient of interest from the introduction of the food to the food supply also is needed (ISLI, 2004, chapter 3).

In many regions of the world, current dietary patterns are not sufficient to achieve the recommended dietary allowance of vitamin D, and supplementation of the diet is encouraged (Holick et al., 2012). Recent estimates conducted for the US population by Bailey et al. (2010) reported that children and adults typically consume, on average, between 5.1 and 7.2 μg (200–300 IU) vitamin D from the diet alone (the RDA is 15 μg/day). With the use of supplement products intakes of 6.6–9.1 μg (264–364 IU) of vitamin D were estimated. Although there is a need for introducing new vitamin D containing foods to the food supply, the use of UV light technology for production of vitamin D containing foods should not be confused with nutrient fortification. Food fortification policies are intended to address nutritional needs within the population, an intent that is achieved through controlled and targeted addition of nutrients to suitable foods that are consumed across multiple age, gender, socioeconomic, and ethnic demographics (*e.g.*, cereals, milk). Mushrooms are a niche food, which are not widely consumed by all population groups (*e.g.*, young children) and are unlikely to achieve the desired population-wide exposure observed with food fortification policies. Consumer awareness of the nutritional importance of vitamin D is increasing as is the desire for natural foods containing vitamin D. The availability of vitamin D mushrooms represents an alternative food source to wild mushrooms, which are harvested on a seasonal basis, and therefore, are not available year round. On a per serving basis, the current levels of vitamin D in UV processed and wild mushrooms available on the marketplace are consistent with those that have been reported in wild mushrooms that have a long-history of safe consumption although data are lacking on patterns of consumption of wild mushrooms. Therefore, a direct comparison of exposure of wild mushrooms to the common button mushroom is not possible. The levels of vitamin D that have been reported for UV processed mushrooms currently on the marketplace are between 3 and 20 μg (120–800 IU)/100 g serving or 2.5–17.5 μg (100–700 IU) per usual 84 g serving. It is highly unlikely that an individual could consume enough of vitamin D mushrooms to exceed the tolerable Upper Limit. For example, based on survey data obtained from the National Center for Health Statistics' (NCHS) National Health and Nutrition Examination Surveys (NHANES), heavy consumers of mushrooms (male adults >20 years of age) in the

Table 3
Dietary reference intakes (DRI) for vitamin D established by the Institute of Medicine (IOM, 2011).

Population subgroup	Age (years)	Recommended dietary allowance (RDA)		Tolerable upper intake level (UL)	
		(μg/day)	(IU/day)	(μg/day)	(IU/day)
Infants	1–3	15	600	62.5	2500
Children and adolescents	4–8	15	600	75	3000
	9–18	15	600	100	4000
Adults	19–70	15	600	100	4000
	>70	20	800	100	4000
Pregnant/lactating	14–50	15	600	100	4000

United States could theoretically consume 34 g of mushrooms per day among 90th percentile consumers of mushrooms and mushroom containing foods (CDC, 2006, 2009; USDA, 2009a). Although such estimates of mushroom consumption are expected to overestimate consumption of foods, (*e.g.*, mushrooms) that are consumed infrequently, the maximum consumption of vitamin D from mushrooms by this population group of heavy mushroom consumers would be 7.15 μg (286 IU) per person day.[1] This vitamin D intake is well below the UL of 100 μg (4000 IU)/person/day for this population group.

7. Discussion

Whole foods do not lend themselves to the standard safety evaluation principles used for food additives and other chemicals, and quantitative assessments of risk for individual whole foods from whatever source cannot be achieved. (WHO, 1987; FAO/WHO, 2002). These limitations of conventional toxicological studies became particularly apparent when animal feeding studies were used to assess the safety of irradiated foods (FAO/WHO, 2002). The application of substantial equivalence within the safety evaluation process of new foods "embodies the concept that if a new food or food component is found to be substantially equivalent to an existing food or food component, it can be treated in the same manner with respect to safety (i.e., the food or food component can be concluded to be as safe as the conventional food or food component." (FAO/WHO, 1996). The FDA has applied the concept of substantial equivalence to the safety assessment of new or modified food substances obtained from new plant varieties and to the safety evaluation of several animal and plant-derived enzyme preparations (62 FR 18937). The FDA also has found the use of substantial equivalence to be applicable to the safety evaluation of UV irradiated foods (42 FR 14635, 65 FR 71057, 61 FR 42383). Similarly, substantial equivalence has been applied to the safety evaluation of vitamin D enhanced mushrooms produced using UV light.

That mushrooms are a natural food source of vitamin D, and that wild mushrooms can contain significant quantities of vitamin D as a result of exposure to ultraviolet radiation from sunlight is widely known. The vitamin D concentration of wild mushrooms is naturally variable; however, vitamin D concentrations in excess of 60 μg (2400 IU) have been reported for samples of wild grown Maitake and Porcini mushrooms (Teichmann et al., 2007; Health Canada, 2012). As recently reported by Phillips et al. (2011), fresh wild mushroom samples obtained from the US marketplace were reported to contain up to 8.4 μg (336 IU) of vitamin D per 100 g of mushrooms on a fresh weight (fw) basis, and commercially available mushrooms processed using UV light technology were reported to contain between 3.3 and 20.9 μg vitamin D (132–836 IU) per 100 g of mushrooms (Phillips et al., 2012). Although the presence of sun-grown mushrooms is limited in the US marketplace, the commercial outdoor cultivation of mushrooms continues to be widely practiced throughout the world, and sun-drying of mushrooms also remains common practice, particularly in Asia, a region where 60% of the Shiitake mushroom supply has been traditionally sold as a dried product (Royse et al., 1985).

Production of vitamin D mushrooms is largely limited to two UV light technologies, the use of high intensity mercury arc lamps that deliver light in the UVB spectrum, or newer technology pulsed light applications which produce high intensity UV light across the UVA through UVC spectrum in a similar profile to that produced by sunlight. It should be noted that wild mushrooms are not exposed to UVC light as this spectrum of light is completely absorbed by ozone in the upper atmosphere; however UVC light has a long-history of safe use in the production of purified and semi-purified food grade sources of vitamin D_2 that are synthesized from ergosterol extracts isolated from *S. cerevisiae* (U.S. FDA, 2012a). UVC irradiation of whole yeast preparations also has been introduced to the food supply for use in bread applications (U.S. FDA, 2007). Finally, early vitamin D fortification efforts for elimination of rickets within the US population were achieved by the safe application of UV irradiation to a large number of foods including milk, butter, and cereal.

Since wild sun-exposed mushrooms have a long-history of safe consumption in the food supply, a comparison of the effects of sunlight on mushroom composition with those occurring in mushrooms processed using UV light was conducted (Simon et al., 2011). Other than the intended effect to increase vitamin D in the mushrooms, no compositional changes of nutritional or toxicological significance were observed (Simon et al., 2011). In addition to the intended increase in vitamin D_2 in the mushrooms, an increase in vitamin D_4 also was identified (Phillips et al., 2012). The presence of vitamin D_4 was attributed to the presence of 22,23-dihydroergosterol in the mushrooms, a putative ergosterol precursor. The levels of vitamin D_4 that have been measured in vitamin D mushrooms processed using UV light are comparable to concentrations that have been measured in wild mushrooms. Overall, the results of compositional testing support the conclusion that the changes imparted to *A. bisporus* mushrooms processed using UV light technology were substantially equivalent to those occurring as a result of sunlight exposure.

The effects of electromagnetic radiation on foods have been a subject of extensive research. Light within the ultraviolet wavelengths of the electromagnetic spectrum represent a form of non-ionizing radiation, which have limited effects on biological molecules, and the corresponding capacity of the technology to impart significant unintended effects on foods is low (Jagger, 1967). Previous experiences during the safety evaluation of ionizing radiation for control microbial growth in food have shown that animal toxicity studies were too insensitive to detect the effects of putative radiolytic products that may be produced at low concentrations in the food. This position is further reflected in FDA's opinion on the generic use of UVC light for germicidal control of microbial

[1] Assumes 700 IU vitamin D per 84 g of mushrooms and daily intake of 34 g of mushrooms from all food sources that may potentially contain vitamin D mushrooms.

growth in food as described in 21 CFR § 179.39 and 21 CFR § 179.40 (U.S. FDA, 2012b). Based on the equivalence of UV and sunlight processing of mushrooms, the history of safe consumption of wild and sun-dried mushrooms, the history of safe use of UV light during food processing (e.g., control of microbial growth and production of vitamin D), and the insensitivity of animals models for use in toxicity testing of whole foods, animal toxicology testing was not considered necessary for the safety assessment of vitamin D mushrooms. Hazard identification and characterization was therefore limited to an evaluation of the compositional changes in mushrooms occurring as a result of the intended use of UV light for production of vitamin D in mushrooms and consideration of the nutritional and toxicological impact of introducing additional dietary sources of vitamins D_2 and D_4 to the food supply.

Studies evaluating the bioavailability of vitamin D mushrooms in humans and animals have been reported. In addition to corroboration of safety, these studies confirm that vitamin D in mushrooms is bioavailable, and mushrooms are a nutritionally relevant food source of vitamin D. Studies evaluating the potency and pharmacokinetics of vitamin D_4 are limited. However, using validated rachitic and vitamin D deficient rodent models, DeLuca and others have reported that vitamin D_4 is one-half to three-quarters as potent as vitamin D_3 (Grab, 1936; McDonald, 1936; Windaus and Trautmann, 1937; Deluca et al., 1968). In addition to being less biologically active, vitamin D_4 displays a lower half life. Based on the long-history of safe consumption of vitamin D_4 in wild mushrooms, the low concentrations produced in mushrooms exposed to UV light, and the lower biological activity and half-life of vitamin D_4 relative to vitamins D_2 and D_3, the presence of vitamin D_4 in the mushrooms was considered nutritionally and toxicologically insignificant.

The recommended dietary allowance for vitamin D in the general population (ages 1 through 70) is 15 μg (600 IU)/person/day. As a result of changes in body weights during ageing, individuals age 71 and older may require higher intakes of up to 20 μg (800 IU)/person/day. The upper limit for vitamin D also been revised upwards, and the IOM concluded that risk of harm from excess dietary vitamin D may increase once intakes surpass 100 μg (4000 IU)/day. Based on current consumption patterns for mushrooms it is difficult to envision that introduction of vitamin D enhanced mushrooms containing concentrations of vitamin D that are comparable to, or lower, than concentrations reported in wild mushrooms and common fish species consumed in the diet (*e.g.*, salmon, tuna) would be of safety concern. The early concerns of Dr. Steenbock in the 1930s that misuse of UV light technology could result in the production of foods with either nutritionally insignificant levels of vitamin D, or levels in excess of those considered safe, should be considered with the intended use of UV light technology for production of vitamin D mushrooms. Published data support that UV light technology has the capacity to produce concentrations of vitamin D in mushrooms that would exceed the safe upper limit for vitamin D. Long-term consumption of mushrooms containing high concentrations of vitamin D resulting in daily intakes of vitamin D above the tolerable upper limit would be undesirable, and could result in toxicity in some consumers. Proper control of vitamin D production should be controlled and can be achieved by the inclusion of UV light processing methods into existing Hazard Analyses and Critical Control Points (HACCP) quality control procedures.

8. Conclusion

Based on the above discussion it can be concluded that the application of UV light technology for production of vitamin D mushrooms is suitable and safe.

Conflict of Interest

R.R.S is an employee of Intertek Cantox. Intertek Cantox has provided scientific consulting services to the U.S. Mushroom Council within the past 3 years. Fees for preparation of this manuscript have been provided to Intertek Cantox by the U.S. Mushroom Council. C.M.W is a member of the Pharmavite Scientific Advisory Board. J.F.B and H.F.D declare no competing interests.

References

Bailey, R.L., Dodd, K.W., Goldman, J.A., Gahche, J.J., Dwyer, J.T., Moshfegh, A.J., Sempos, C.T., Picciano, M.F., 2010. Estimation of total usual calcium and vitamin D intakes in the United States. J. Nutr. 140, 817–822.

Bills, C.E., 1938. The chemistry of vitamin D. J. Am. Med. Assoc. 110, 2150–2155.

Bills, C.E., Honeywell, E.M., Wirick, A.M., Nussmeier, M., 1931. A critique of the line test for vitamin D. J. Biol. Chem. 90, 619–636.

Boomsma, F., Jacobs, H.J.C., Havinga, E., van der Gen, A., 1977. The "overirradiation products" of previtamin D and tachysterol: toxisterols. Recueil des Travaux Chimiques des Pays-Bas 96 (4), 104–112.

Braun, M.W., Fuß, W., Kompa, K.L., Wolfrum, J., 1991. Improved photosynthesis of previtamin D by wavelengths of 280–300 nm. J. Photochem. Photobiol. A Chem. 61 (1), 15–26.

Calvo, M.S., Babu, U.S., Garthoff, L.H., Woods, T.O., Dreher, M., Hill, G., Nagaraja, S., 2012. Vitamin D(2) from light-exposed edible mushrooms is safe, bioavailable and effectively supports bone growth in rats. Osteoporos. Int. (Epub ahead of print, February 23).

CDC (2006). Analytical and Reporting Guidelines: The National Health and Nutrition Examination Survey (NHANES). Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS), Hyattsville, MD. <http://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/nhanes_analytic_guidelines_dec_2005.pdf>.

CDC (2009). National Health and Nutrition Examination Survey (NHANES): 2005–2006. Centers for Disease Control and Prevention (CDC), National Center for Health Statistics (NCHS), Hyattsville (MD). <http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/nhanes05_06.htm>.

Chesney, R.W., 1989. Vitamin D: can an upper limit be defined? J. Nutr. 119, 1825–1828.

Constable, A., Jonas, D., Cockburn, A., Davi, A., Edwards, G., Hepburn, P., Herouet-Guicheney, C., et al., 2007. History of safe use as applied to the safety assessment of novel foods and foods derived from genetically modified organisms. Food Chem. Toxicol. 45 (12), 2513–2525.

DeLuca, H.F., 2004. Overview of general physiologic features and functions of vitamin D. Am. J. Clin. Nutr. 80, 1689S–1696S.

DeLuca, H.F., Weller, M., Blunt, J.W., Neville, P.F., 1968. Synthesis, biological activity, and metabolism of 22, 23-3H-vitamin D4. Arch. Biochem. Biophys. 124, 122–128.

EC, 1997. Regulation EC No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients [L43]. Off. J. Eur. Communities 40, 1–6. <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:1997R0258:20090120:EN:PDF> (Amended to 2009).

FAO/WHO, 1996. Biotechnology and Food Safety. Report of a Joint FAO/WHO Consultation, September 30–October 4, 1996, Rome, Italy. Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO), Geneva, Switz. FAO Food and Nutrition Paper No. 61. <ftp.fao.org/es/esn/food/biotechnology.pdf>.

FAO/WHO, 2002. Vitamin D. In: Human Vitamin and Mineral Requirements. Report of a Joint FAO/WHO Expert Consultation, Bangkok, Thailand. Food and Agricultural Organization of the United Nations (FAO)/World Health Organization (WHO), Rome, Italy, pp. 109–120. <ftp://ftp.fao.org/docrep/fao/004/y2809e/y2809e08.pdf>.

U.S. FDA, 1997. Substances generally recognized as safe: proposed rule (21 CFR Parts 170, 184, 186, and 570) [Docket No. 97N–0103]. Fed. Regist. (US) 62, 18937–18964.

FSANZ, 2007. Safety Assessment of Genetically Modified Foods Guidance Document. Foods Standards Australia New Zealand (FSANZ), Canberra, Australia/Wellington, NZ. <http://www.foodstandards.gov.au/_srcfiles/GM%20FINAL%20Sept%2007L%20_2_pdf>.

Grab, W., 1936. Bie auswertung der antirachitischen Wirksamkeit neuer sterinderivate im versuch an ratten und kuken. Z. Physiol. Chem. 243, 63–89.

Havinga, E., 1973. Vitamin D, example and challenge. Experientia 29, 1181–1193.

Havinga, E., de Kock, R.J., Rappoldt, M.P., 1960. The photochemical interconversions of provitamin D, lumisterol, previtamin D and tachysterol. Tetrahedron 11(4), 276–284.

Health Canada, 2006. Guidelines for the Safety Assessment of Novel Foods. Health Canada, Food Directorate, Health Products and Food Branch, Ottawa, Ont. <http://www.hc-sc.gc.ca/fn-an/legislation/guide-ld/nf-an/guidelines-lignesdirectrices-eng.php>.

Health Canada, 2012. Canadian Nutrient File (CNF). Health Canada, Nutrition Research Division Bureau of Nutritional Sciences, Food Directorate, Health Protection Branch (HPB), Ottawa, Ont. <http://www.hc-sc.gc.ca/fn-an/nutrition/

fiche-nutri-data/index-eng.php> (date modified 02.10.02.12; last accessed in 2009).
Heaney, R.P., Recker, R.R., Grote, J., Horst, R.L., Armas, L.A., 2011. Vitamin D(3) is more potent than vitamin D(2) in humans. J. Clin. Endocrinol. Metab. 96, E447–E452.
Holick, M.F., 2007. Vitamin D deficiency. N. Engl. J. Med. 357, 266–281.
Holick, M.F., Binkley, N.C., Bischoff-Ferrari, H.A., Gordon, C.M., Hanley, D.A., Heaney, R.P., Murad, M.H., Weaver, C.M., 2012. Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited. J. Clin. Endocrinol. Metab. 97(4), 1153–1158 (Epub 2012 March 22).
ILSI, 2004. Nutritional and safety assessments of foods and feeds nutritionally improved through biotechnology: an executive summary a Task Force Report by the International Life Sciences Institute, Washington, DC. Comp. Rev. Food Sci. Food Saf. 3, 35–104.
ILSI, 2008. Evaluation of protein safety in the context of agricultural biotechnology [ILSI International Food Biotechnology Committee Task Force on Protein Safety]. Food Chem. Toxicol. 46, S71–S97.
IOM, 1997. Vitamin D. In: Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. National Academy of Sciences, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine (IOM). National Academy Press (NAP), Washington, DC, pp. 250–287. <http://www.nap.edu/openbook.php?record_id=5776&page=250>.
IOM, 2011. Dietary Reference Intakes for Calcium and Vitamin D. National Academy of Science (NAS), Institute of Medicine (IOM), Food and Nutrition Board, Washington, DC. <http://www.nap.edu/catalog.php?record_id=13050>.
Jagger, J., 1967. Introduction to Research in Ultraviolet Photobiology. Prentice-Hall, Inc., Englewood Cliffs, NJ.
JAMA, 1931. The vitamin content of mushrooms. J. Am. Med. Assoc. 96, 1637–1638.
Jasinghe, V.J., Perera, C.O., Barlow, P.J., 2006. Vitamin D2 from irradiated mushrooms significantly increases femur bone mineral density in rats. J. Toxicol. Environ. Health A 69, 1979–1985.
JFCD, 2004. [Japanese] Food Composition Database. Sugiyama Jogakuen University. <http://database.food.sugiyama-u.ac.jp/index_asia.php> and <http://database.food.sugiyama-u.ac.jp/PC_ASIA/search2.php>.
Kalaras, M.D., Beelman, R.B., Elias, R.J., 2012. Effects of postharvest pulsed UV light treatment of white button mushrooms (*Agaricus bisporus*) on vitamin D2 content and quality attributes. J. Agric. Food Chem. 60, 220–225.
Koyyalamudi, S.R., Jeong, S.-C., Song, C.-H., Cho, K.Y., Pang, G., 2009. Vitamin D_2 formation and bioavailability from *Agaricus bisporus* button mushrooms treated with ultraviolet irradiation. J. Agric. Food Chem. 57, 3351–3355.
Kristensen, H.L., Rosenqvis, E., Jakobsen, J., 2012. Increase of vitamin D_2 by UV-B exposure during the growth phase of white button mushroom (*Agaricus bisporus*). Food Nutr. Res. 56, 7114.
Lee, G.S., Byun, H.S., Yoon, K.H., Lee, J.S., Choi, K.C., Jeung, E.B., 2009. Dietary calcium and vitamin D2 supplementation with enhanced Lentinula edodes improves osteoporosis-like symptoms and induces duodenal and renal active calcium transport gene expression in mice. Eur. J. Nutr. 48, 75–83.
Lu, Z., Chen, T.C., Zhang, A., Persons, K.S., Kohn, N., Berkowitz, R., Martinello, S., Holick, M.F., 2007. An evaluation of the vitamin D3 content in fish: is the vitamin D content adequate to satisfy the dietary requirement for vitamin D? J. Steroid Biochem. Mol. Biol. 103, 642–644.
Lunde, G., Aschdhoug, V., Kringstad, H., 1937. Fish as a source of vitamins A and D: herring, brisling, and their products. Ind. Eng. Chem. 29, 1171–1175.
Mattila, P.H., Piironen, V.I., Uusi-Rauva, E.J., Koivistoinen, P.E., 1994. Vitamin D contents in edible mushrooms. J. Agric. Food Chem. 42, 2449–2453.
McDonald, F.G., 1936. The multiple nature of vitamin D. III. irradiated 22-dihydroergosterol. J. Biol. Chem. 114, lxv.
Munro, I.C., McGirr, L.G., Nestmann, E.R., Kille, J.W., 1996a. Alternative approaches to the safety assessment of macronutrient substitutes. Regul. Toxicol. Pharmacol. 23, S-S14.
Munro, I.C., Ford, R.A., Kennepohl, E., Sprenger, J.G., 1996b. Correlation of structural class with no-observed-effect levels: a proposal for establishing a threshold of concern. Food Chem. Toxicol. 34, 829–867.
NIFES, 2010. [Website]. National Institute of Nutrition and Seafood Research (NIFES), Bergen, Norway. <http://www.nifes.no/index.php?page_id=&lang_id=2>.
OECD, 2007. Consensus Document on Compositional Considerations for New Varieties of the Cultivated Mushroom *Agaricus bisporus*: Key Food and Feed Nutrients, Anti-nutrients and Toxicants. Organisation for Economic Co-operation and Development (OECD), Environment Directorate, Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology, Paris, France. Series on the Safety of Novel Foods and Feeds, No. 15, ENV/JM/MONO(2007)5. <http://www.olis.oecd.org/olis/2007doc.nsf/LinkTo/NT00000E7A/$FILE/JT03225679.PDF>.
Ostermeyer, U., Schmidt, T., 2006. Vitamin D and provitamin D in fish: determination by HPLC with electrochemical detection. Eur. Food Res. Technol. 222, 403–413.
Outila, T.A., Mattila, P.H., Piironen, V.I., Lamberg-Allardt, C.J.E., 1999. Bioavailability of vitamin D from wild edible mushrooms (*Cantharellus tubaeformis*) as measured with a human bioassay. Am. J. Clin. Nutr. 69, 95–98.
Ozzard, A., Hear, G., Morrison, G., Hoskin, M., 2008. Vitamin D deficiency treated by consuming UVB-irradiated mushrooms. Br. J. Haematol. 58, 644–645.
Pauli, G.H., Takeuchi, C.A., 1986. Irradiation of foods – an FDA perspective. Food Rev. Int. 2, 79–107.
Phillips, K.M., Ruggio, D.M., Horst, R.L., Minor, B., Simon, R.R., Feeney, M.J., Byrdwell, W.C., Haytowitz, D.B., 2011. Vitamin D and sterol composition of 10 types of mushrooms from retail suppliers in the United States. J. Agric. Food Chem. 59, 7841–7853.
Phillips, K.M., Horst, R.L., Koszewski, N.J., Simon, R.R., 2012. Vitamin D4 in mushrooms. PLoS ONE 7 (8), e40702.
Plum, L.A., DeLuca, H.F., 2010. Vitamin D, disease and therapeutic opportunities. Nat. Rev. Drug. Discov. 9 (12), 941–955.
Rajakumar, K., Greenspan, S.L., Thomas, S.B., Holick, M.F., 2007. Solar ultraviolet radiation and vitamin D. Am. J. Publ. Health 97, 1746–1754.
Royse, D.J., Schisler, L.C., Diehle, D.A., 1985. Shiitake mushrooms: consumption, production and cultivation. Interdiscip. Sci. Rev. 10, 329–335.
Schneider, H.A., 1973. Harry Steenbock (1886–1967) – a biographical sketch. J. Nutr. 103 (9), 1233–1247.
Simon, R.R., Phillips, K.M., Horst, R.L., Munro, I.C., 2011. Vitamin D mushrooms: comparison of the composition of button mushrooms (*Agaricus bisporus*) treated postharvest with UVB light or sunlight. J. Agric. Food Chem. 59, 8724–8732.
Stephensen, C.B., Zerofsky, M., Burnett, D.J., Lin, Y.P., Hammock, B.D., Hall, L.M., McHugh, T., 2012. Ergocalciferol from mushrooms or supplements consumed with a standard meal increases 25 hydroxyergocalciferol but decreases 25-hydroxycholecalciferol in the serum of healthy adults. J. Nutr. 142 (7), 1246–1252 (Epub 2012 May 23).
Teichmann, A., Dutta, P.C., Staffas, A., Jagerstäd, M., 2007. Sterol and vitamin D2 concentrations in cultivated and wild grown mushrooms: effect of UV radiation. Lebensm. Wiss. Technol. 40, 815–822.
U.S. FDA, 1992. Statement of policy: foods derived from new plant varieties: notice [Docket No. 92N-0139]. Fed. Regist. (US) 57, 22983–23005.
U.S. FDA, 2007. Letter from Geraldine A. June, CFSAN to Dennis Gordon, Lallemand Inc. [Authorizing the Addition of Vitamin D Enriched UVC Irradiated Yeast to Bread]. U.S. Food and Drug Administration (U.S. FDA), Center for Food Safety and Applied Nutrition (CFSAN), Office of Nutritional Products, Labeling and Dietary Supplements, College Park, MD.
U.S. FDA, 2012a. Part 184—Direct food substances affirmed as generally recognized as safe. Section §184.1950—Vitamin D. In: U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration). U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO), Washington, DC. <http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR>.
U.S. FDA, 2012b. Part 179—Irradiation in the production, processing and handling of food. Sections §179.39—ultraviolet radiation for the processing and treatment of food, §179.41—pulsed light for the treatment of food. In: U.S. Code of Federal Regulations (CFR). Title 21: Food and Drugs (U.S. Food and Drug Administration). U.S. Food and Drug Administration (U.S. FDA), U.S. Government Printing Office (GPO), Washington, DC. <http://www.gpo.gov/fdsys/browse/collectionCfr.action?collectionCode=CFR>.
Urbain, P., Singler, F., Ihorst, G., Biesalski, H.K., Bertz, H., 2011. Bioavailability of vitamin D2 from UV-B-irradiated button mushrooms in healthy adults deficient in serum 25-hydroxyvitamin D: a randomized controlled trial. Eur. J. Clin. Nutr. 65, 965–967.
USDA, 2008. Search results for: cod liver oil, Wild Atlantic Salmon, Channel Catfish Wild, Atlantic Herring, Eastern Oyster, Tofu [Vitamin D content]. In: USDA National Nutrient Database for Standard Reference, Release 21. Prepared by U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Human Nutrition Research Center Nutrient Data Laboratory, Beltsville, MD. <http://ndb.nal.usda.gov/ndb/foods/list>.
USDA, 2009a. Search results for: Maitake [Vitamin D content]. In: USDA National Nutrient Database for Standard Reference, Release 22. Prepared by U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Human Nutrition Research Center Nutrient Data Laboratory, Beltsville, MD. <http://ndb.nal.usda.gov/ndb/foods/list>.
USDA, 2012. Search results for: mushroom and ultraviolet [vitamin D content]. In: USDA National Nutrient Database for Standard Reference, Release 25. Prepared by U.S. Department of Agriculture (USDA), Agricultural Research Service (ARS), Human Nutrition Research Center Nutrient Data Laboratory, Beltsville, MD. <http://ndb.nal.usda.gov/ndb/foods/list>.
Velluz, L., Amiard, G., Petit, A., 1949. Le precalciferol: ses relations d'equilibre avec le calciferol. Soc. Chim. Fr. 16, 401–507.
Wang, Y., Zhu, J., DeLuca, H.F., 2012. Where is the vitamin D receptor? Arch. Biochem. Biophys. 523 (1), 123–133 (Epub 2012 April 6).
WARF, 2012. Steenbock and WARF's Founding. <http://www.warf.org/about/index.jsp?cid=26&scid=33>.
WHO, 1987. Principles for the Safety Assessment of Food Additives and Contaminants in Food. World Health Organization (WHO), International Programme on Chemical Safety (IPCS), Geneva, Switz. Environmental Health Criteria, No. 70. <http://www.inchem.org/documents/ehc/ehc/ehc70.htm>.
Windaus, A., Trautmann, Z., 1937. Ueber das krystallisierte Vitamin D_4. Z. Physiol. Chem. 247, 185–188.