Plant Oils as Potential Sources of Vitamin D

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To combat vitamin D insufficiency in a population, reliable diet sources of vitamin D are required. The recommendations to consume more oily fish and the use of UVB-treated yeast are already applied strategies to address vitamin D insufficiency. This study aimed to elucidate the suitability of plant oils as an alternative vitamin D source. Therefore, plant oils that are commonly used in human nutrition were first analyzed for their content of vitamin D precursors and metabolites. Second, selected oils were exposed to a short-term UVB irradiation to stimulate the synthesis of vitamin D. Finally, to elucidate the efficacy of plant-derived vitamin D to improve the vitamin D status, we fed UVB-exposed wheat germ oil (WGO) for 4 weeks to mice and compared them with mice that received non-exposed or vitamin D₃ supplemented WGO. Sterol analysis revealed that the selected plant oils contained high amounts of not only ergosterol but also 7-dehydrocholesterol (7-DHC), with the highest concentrations found in WGO. Exposure to UVB irradiation resulted in a partial conversion of ergosterol and 7-DHC to vitamin D₂ and D₃ in these oils. Mice fed the UVB-exposed WGO were able to improve their vitamin D status as shown by the rise in the plasma concentration of 25-hydroxyvitamin D [25(OH)D] and the liver content of vitamin D compared with mice fed the non-exposed oil. However, the plasma concentration of 25(OH)D of mice fed the UVB-treated oil did not reach the values observed in the group fed the D₃ supplemented oil. It was striking that the intake of the UVB-exposed oil resulted in distinct accumulation of vitamin D₂ in the livers of these mice. In conclusion, plant oils, in particular WGO, contain considerable amounts of vitamin D precursors which can be converted to vitamin D via UVB exposure. However, the UVB-exposed WGO was less effective to improve the 25(OH)D plasma concentration than a supplementation with vitamin D₃.

Keywords: ergosterol, 7-dehydrocholesterol, vitamin D, plant oils, wheat germ oil, ultraviolet light irradiation, bioavailability, mice

INTRODUCTION

Food sources of vitamin D are scarce. Although oily fish is considered to be a good source of vitamin D₃ (1, 2), its consumption and its vitamin D content is not high enough to significantly improve the vitamin D status of humans (3). Besides fish, mushrooms are often considered as another valuable source of vitamin D, in particular of vitamin D₂. However, the major natural vitamin D metabolite in fungi and yeast is the vitamin D precursor ergosterol, which can be converted to vitamin D₂ via UVB exposure. However, the UVB-exposed WGO was less effective to improve the 25(OH)D plasma concentration than a supplementation with vitamin D₃.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; 7-DHC, 7-dehydrocholesterol; LLOQ, lower limit of quantification; WGO, wheat germ oil.
by UVB irradiation (4). The UVB-exposed baker’s yeast, which has been approved by the European Food Safety Authority as a reliable ingredient to enrich bakery products with vitamin D, is a prominent example for a successful application of UVB irradiation to enhance vitamin D in natural foods (5). However, less data are available on vitamin D precursors and metabolites in plants. Yellow oat grass (Trisetum flavescens) is well described for its capability to synthesize bioactive vitamin D. It contains vitamin D glycosides which can be hydrolyzed in the gut or by the gastrointestinal microflora to the biologically active 1,25-dihydroxyvitamin D (6–8). Other so-called calcinogenic plants that contain active vitamin D forms are Solanum malacoxylon, Cestrum diurnum, and Nierembergia veitchii of the Solanaceae family (6–8). These plants are presumed to cause calcinosis in grazing animals due to the hypercalcemic effect of toxic 1,25-dihydroxyvitamin D levels (9). Vitamin D metabolites were also found in Cucurbitaceae, Fabaceae, and Poaceae (10–12). Besides that, certain plants are associated with fungal endophytes (13, 14) or are capable to produce the vitamin D precursor 7-dehydrocholesterol (7-DHC) on its own via the lanosterol pathway (15). Based on these data, we hypothesized that plant oils could also contain vitamin D precursors or metabolites. The main aims of this investigation were [1] to identify and quantify precursors and metabolites of vitamin D in plant oils that are used in human nutrition and [2] to investigate whether a short-term exposure of selected oils to UVB light could increase their vitamin D content. To elucidate possible adverse effects of UVB exposure on the quality of the oils, we analyzed oxidative biomarkers and tested the sensory quality of the UVB-exposed oils. Additional tests were conducted to assess the stability of these vitamin D metabolites subsequent to thermal treatment and storage of the UVB-exposed oil. Finally, we aimed to elucidate the efficacy of plant-derived vitamin D to improve the vitamin D status by feeding an UVB-exposed plant oil to mice.

MATERIALS AND METHODS

Characterization of Vitamin D Metabolites in the Plant Oils

Avocado oil, linseed oil, olive oil, pumpkinseed oil, rapeseed oil, soya oil, sunflower oil, and wheat germ oil (WGO) were used to characterize and quantify their vitamin D precursors and metabolites. From each type of oil, three commercially available representatives were obtained from local supermarkets and used for the analyses. The oil samples selected for analyses were flushed with N2 after the first opening, to avoid oxidation processes and treatment of the UVB-exposed oil. Finally, we aimed to elucidate the efficacy of plant-derived vitamin D to improve the vitamin D status by feeding an UVB-exposed plant oil to mice.

UVB Exposure of Selected Oils

Rapeseed oil, avocado oil, and WGO were used for the UVB treatments and exposed to UVB light. In the first approach, aliquots of the three oils were placed into plastic vessels (thickness of the oil layer 1.0 mm) and exposed to UVB light for 0 (control), 4, and 8 min at room temperature. The UVB-emitting lamp (650 μW/cm², in a distance of 15 cm, UV-8M, Heroloba GmbH, Wiesloch, Germany) was placed 10 cm above the oil surface. During that treatment, the oils were flushed with N2. In a second approach, WGO was used to investigate the impact of the oil layer thickness on the efficacy of vitamin D formation through UVB irradiation. Therefore, different volumes of the oil were filled in glass vessels to reach a layer thickness of either 1.6 or 3.2 mm, to be UVB-exposed for 10 min at room temperature. During that time, the oil samples were constantly stirred by a magnetic stirrer under N2. The oil samples were stored at −20°C until analyses of vitamin D3, vitamin D2, and tocopherols. In addition to that, the peroxide and the acid values were analyzed in the 10 min UVB-exposed WGO and compared with those of the non-exposed oil of the same batch. The analyses were complemented by organoleptic tests. The UVB-treated oil (exposure time: 10 min, oil layer thickness: 3.2 mm) which was intended for use in the mouse study was analyzed for vitamin D metabolites and stored at −20°C until preparation of the diet. All diets were stored at −20°C until their administration.

Thermal Treatment and Storage of UVB-Exposed Wheat Germ Oil

To estimate the stability of the UVB-exposed oils, aliquots of the 10-min UVB-exposed WGO (1.6 mm layer) were (1) heated at 100 or 180°C for 10 min and (2) stored for 1 day, 2 weeks, or 4 weeks at room temperature in the dark. After the thermal treatment and storage terms, the WGOs were flushed with N2 and stored at −20°C until further analysis. Aliquots of untreated oil samples of the same batch were used as a reference. Besides the concentration of the vitamin D metabolites, the concentration of tocopherols were analyzed to gain information about oxidation processes. The thermally treated and the 4 weeks-stored WGOs were analyzed to record the peroxide and the acid values and subjected to organoleptic tests.

Analysis of Vitamin D Metabolites in Oils

The concentrations of ergosterol, 7-DHC, vitamin D3, and vitamin D2 were analyzed by high performance liquid chromatography (HPLC) coupled with a tandem mass spectrometry system (LC-MS/MS), as described elsewhere (16). In brief, aliquots of the oils (300 mg) were mixed with internal deuterated standards (7-DHC-d6, Chemaphor Incorporation, Ottawa, ON, Canada; vitamin D3–d3, vitamin D2–d3, Sigma Aldrich Chemie GmbH, Taufkirchen, Germany). After an overnight hydroslysis, the D vitamins were extracted with n-hexane, washed with ultrapure water, solved in n-hexane/isopropanol (99/1, v/v), and fractionated using an Agilent 1100 HPLC-System with a LiChrospher Si 60 column (250 mm × 4.0 mm, 5 μm particle size; Agilent Technologies, Waldbronn, Germany) (17). Subsequent to drying the fractions, 100 μl of 4-phenyl-1,2,4-triazoline-3,5-dione (0.15 mg/ml acetonitrile, Sigma Aldrich) were added for derivatization (18). After evaporation of the solvent, the samples were resolved in methanol, mixed with 10 mM ammonium formate solution (4/1, v/v), and analyzed using HPLC (Agilent 1100) with a Hypersil ODS-column (150 mm × 2.0 mm, 5 μm particle size; VDS Optilab Chromatographic Technik GmbH, Berlin, Germany) coupled with a MS system (API 2000, Sciex, Darmstadt, Germany). Two mixtures with a gradient flow were used as mobile phase (A: 1 mM methylamine in acetonitrile; B: 1 mM methanol and 5 mM ammonium formate in acetonitrile/ultrapure water, 1/1, v/v). External standards (ergosterol, 7-DHC, vitamin D3, vitamin D2, Sigma Aldrich) were used for calibration. The multiple reaction monitoring (MRM) was used.
for quantification at the following mass transitions: ergosterol, 603 > 377; 7-DHC, 591 > 365; 7-DHC-d6, 597 > 371; vitamin D$_{2}$, 603 > 298; vitamin D$_{1}$–d$_{6}$, 606 > 301; vitamin D$_{3}$, 591 > 298 and vitamin D$_{2}$–d$_{6}$, 594 > 301.

Except for the analysis of the vitamin D metabolites in rape-seed, avocado, and WGO after UVB exposure (1.0 mm layer), all analyzes were run in duplicate. The lower limit of quantification (LLOQ, signal-to-noise ratio = 10) in oils was 4.3 ng/g for all vitamin D metabolites. The coefficients of variation were 10.1% for ergosterol, 12.8% for 7-DHC, 5.9% for vitamin D$_{2}$, and 9.2% for vitamin D$_{3}$ (n = 10).

**Analysis of Autoxidation Markers in Oils**

The concentrations of the tocopherols were measured by a modified HPLC method of Coors (19). Prior to the quantification of the tocopherols, aliquots of the oils were solved in n-hexane (1/100, w/v), mixed thoroughly, and separated isocratically by HPLC (Agilent 1100) using a LiChrospher Si 60 column (250 mm × 4.0 mm, 5 µm particle size; Agilent Technologies). A mixture of n-hexane and isopropanol (99/1, v/v) was used as mobile phase (flow rate: 1 ml/min). The α-, β-, and γ-tocopherols were detected by a fluorescent detector (emission: 330 nm, excitation: 295 nm). External standards (α-, β-, γ-tocopherols, Supelco, Bellefonte, PA, USA) were used for calibration. The peroxide and the acid values of the oils were determined according to the German official methods (20, 21).

**Organoleptic Characterization of the Oils**

The UVB-exposed, the thermally treated, the 4 weeks-stored, and the -untreated WGOs were evaluated by a trained panel (ÖHMI Analytik GmbH, Magdeburg, Germany) in a blinded fashion. Taste, aroma, color, and transparency of the oils were judged at 40°C (22), and the oils were ranked according to its organoleptic quality (23).

**Mouse Study**

The experimental procedures described below followed the established guidelines for the care and handling of laboratory animals according to the National Research Council (24) and were approved by the local government (Landesverwaltungsamt Sachsen-Anhalt, Germany; approval number 42502-5-34). All mice were housed in pairs on a 12-h light, 12-h dark cycle in a room controlled for temperature (22 ± 2°C) and relative humidity (50–60%). Food and water were provided ad libitum.

Forty-two 4-week-old male mice (C57BL/6NCrI, Charles River Laboratories, Sulzfeld, Germany) were used. Five weeks prior to the actual treatment, the mice received a vitamin D-free semi-synthetic basal diet (20% casein, 20% sucrose, 38.8% starch, 10% WGO, 6% vitamin-mineral-mixture, 5% cellulose, and 0.2% DL-methionine) to reduce their vitamin D status. Except for the vitamin D, all other vitamins and minerals were supplemented according to the recommendations of the AIN (25). After the 5-week, six mice were sacrificed to determine the vitamin D status of these animals at baseline. The remaining 36 mice (mean body weight: 13.9 ± 0.8 g) were allotted to 3 groups of 12 mice each and fed the basal vitamin D-free diet with 10% of either the 10 min UVB-exposed WGO (3.2 mm-layer, WGO-UV), the untreated WGO, or WGO that was supplemented with synthetic vitamin D$_{3}$ (WGO-D$_{3}$) in comparable amounts to the total vitamin D content in the UVB-exposed oil. In the experimental diet fed to the WGO-UV group, a mean vitamin D$_{3}$ concentration of 87.3 µg/kg diet was measured, whereas no vitamin D$_{1}$ was found. The diet fed to the WGO-D$_{3}$ group had a mean analyzed vitamin D$_{3}$ concentration of 80.0 µg/kg and no vitamin D$_{2}$, while in the diet fed to the WGO group neither vitamin D$_{2}$ nor vitamin D$_{3}$ could be detected. The experimental diets were fed to the mice for 4 weeks. Individual body weights and mean food intake per cage were recorded weekly. Finally, the mice were sacrificed after a 4-h food deprivation under light anesthesia with diethyl ether. Blood was collected into heparin tubes (Sarstedt, Nümbrecht, Germany). Plasma was separated by centrifugation at 3000 x g at 4°C for 20 min and stored at −20°C until analysis of the vitamin D metabolites. The livers were harvested, immediately snap-frozen in liquid N$_{2}$, and stored at −80°C until analysis of the vitamin D metabolites.

**Analysis of Vitamin D Metabolites in Plasma, Diet, and Liver**

The plasma concentrations of ergosterol, 7-DHC, vitamin D$_{2}$, vitamin D$_{3}$, 25-hydroxyvitamin D [25(OH)D], in particular 25(OH)D$_{2}$ and 25(OH)D$_{3}$, were measured using LC-MS/MS (16). Plasma aliquots (100 µl) were mixed with potassium hydroxide, ascorbic acid, sodium sulfide, and internal standards (7-DHC-d$_{6}$, vitamin D$_{2}$–d$_{6}$, vitamin D$_{3}$–d$_{6}$, and 25(OH)D$_{2}$–d$_{6}$, Chemaphor Incorporation) and flushed with N$_{2}$. After incubation for 3 h at 37°C, followed by 17 h incubation at room temperature, the vitamins were extracted two times with n-hexane and mixed with 4-phenyl-1,2,4-triazoline-3,5-dione for derivatization. Subsequently, the extracts were dried, resolved in methanol and 10 mM ammonium formate (4/1, v/v), and analyzed via LC-MS/MS as described before for the oils samples. Mass transitions for the hydroxylated metabolites were 25(OH)D$_{2}$, 519 > 298; 25(OH)D$_{3}$, 567 > 298; and 25(OH)D$_{2}$–d$_{6}$, 613 > 298. In plasma samples, the LLOQ of ergosterol, vitamin D$_{2}$, and vitamin D$_{3}$ was 1.25 nmol/l, that of 25(OH)D$_{2}$ and 25(OH)D$_{3}$ was 4.2 nmol/l.

The vitamin D$_{2}$ and D$_{3}$ concentrations in the diets and liver samples were analyzed as already described for the oil samples. The vitamin D$_{2}$ and vitamin D$_{3}$ concentrations of the diets were analyzed in aliquots of 1 g in triplicate. In the diets, the LLOQ for both vitamin D metabolites was 4.3 ng/g. Liver aliquots of 200 mg were analyzed for their concentrations of ergosterol, 7-DHC, vitamin D$_{2}$, vitamin D$_{3}$, 25(OH)D$_{2}$, and 25(OH)D$_{3}$. In the liver samples, the LLOQ was 5.0 ng/g for vitamin D$_{2}$, 10.5 ng/g for vitamin D$_{3}$, 0.3 ng/g for 25(OH)D$_{2}$, and 2.1 ng/g for 25(OH)D$_{3}$.

**Analysis of Tocopherols in Plasma**

To analyze the α-tocopherol concentrations in plasma, aliquots (30 µl) were mixed with pyrogallol solution (1% in ethanol, absolute) and saturated sodium hydroxide solution for hydrolysis. Subsequently, the samples were incubated at 70°C for 30 min, and tocopherols were extracted with n-hexane and ultrapure water. The supernatant was directly applied to the HPLC (26). HPLC conditions were the same as described for the tocopherol analysis of the oils.
Statistical Analysis
Data concerning the characterization of the plant oils were not subjected to statistical analysis. Values of the in vivo experiment are presented as means ± SD. If values were below the LLOQ, randomly generated values (between 0 and the appropriate LLOQ) were used for statistical treatment analyses. Statistical analyses were conducted using SPSS statistical software (SPSS 22, IBM; Armonk, NY, USA). All data were subjected to a normality test using the Shapiro–Wilk test. If the data followed a normal distribution, differences between the groups were analyzed by one-way analysis of variances (ANOVA), and subsequently subjected to the Levene’s test for homoscedasticity. In case of homogeneity of variance, the three treatment groups were compared by the Tukey’s test, in case of unequal variances by the Games–Howell test. If the data were not normal distributed, the Kruskal–Wallis test was used to analyze differences between the groups and the Mann–Whitney U test was conducted for post hoc comparisons of the three treatment groups (corrected by Bonferroni). Differences were considered to be significant at $P < 0.05$.

RESULTS
Vitamin D and Vitamin D Precursors in Selected Plant Oils
Eight commercially available plant oils for human nutrition were characterized for their vitamin D precursors and vitamin D contents. Analysis revealed that the concentrations of the vitamin D precursors ergosterol and 7-DHC varied strongly between the different oils, but all oils had a markedly higher concentration of ergosterol than of 7-DHC (Figure 1). The highest ergosterol concentration was found in the WGOs (22.1–34.2 μg/g) followed by the avocado oils (4.2–23.4 μg/g) and the sunflower oils (7.9–17.4 μg/g). Oils derived from rapeseed, soya, and linseed had lower ergosterol concentrations that ranged from 4.1 to 9.5 μg/g; the lowest concentrations were found in olive and pumpkinseed oils (<4.5 μg/g). Analyses revealed that the WGOs had the highest concentrations of 7-DHC (638–669 ng/g), while other oils had very low quantities of 7-DHC (Figure 1). The 7-DHC concentration in the linseed oils ranged between 41.1 and 95.7 ng/g; the other oils had 7-DHC concentrations between 10.7 and 47.9 ng/g. Vitamin D$_1$ and D$_3$ were not quantifiable in the eight analyzed plant oils.

Formation of Vitamin D in the UVB-Exposed Oils
To elucidate the impact of a short-term UVB irradiation on the formation of vitamin D in the plant oils, we exposed rapeseed oil, avocado oil, and WGO that differed widely in their amounts of vitamin D precursors to UVB light. The UVB exposure of rapeseed, avocado, and WGO increased the vitamin D concentrations in these oils in a time-dependent manner (Figure 2). The amount of vitamin D$_2$ produced by UVB irradiation was higher in the wheat germ and the avocado oil than in the rapeseed oil. The amount of the vitamin D$_3$ increased only in the WGO upon UVB exposure, but not in the rapeseed and the avocado oil, which was probably due to the higher 7-DHC concentration in the WGO (Figure 2). The data further showed a significant impact of the layer thickness on the efficacy of the UVB exposure to increase the vitamin D content. The concentrations of vitamin D$_1$ and vitamin D$_3$ in the 1.6 mm-layer of WGO, which was UVB-exposed for 10 min, were 1035 and 37.0 ng/g, respectively. UVB-exposed WGO with a 3.2 mm-oil layer thickness had still high concentrations of vitamin D$_1$ and vitamin D$_3$, reaching 82 and 94% of the concentrations observed in the 1.6 mm-oil layer.

Changes in Quality Parameters of the Oils upon UVB Exposure
To elucidate the impact of the UVB treatment on the oil quality, the tocopherol concentrations and markers of autoxidation were measured in the UVB-exposed oils. The tocopherol concentrations of the 8-min UVB-exposed wheat germ and avocado oil (1.0 mm-layer) were not different from those of the untreated
TABLE 1 | Influence of UVB exposure* on tocopherol concentrations of selected plant oils.

| Oil           | UVB exposurea time (min) | Tocopherols (mg/100 g) |                |                |
|---------------|-------------------------|------------------------|-----------------|----------------|
|               |                        | α          | β          | γ          |
| Rapeseed      | 0                       | 19.1       | 0          | 36.8       |
|               | 4                       | 18.7       | 0          | 36.4       |
|               | 8                       | 16.8       | 0          | 36.0       |
| Avocado       | 0                       | 20.8       | 0.8        | 1.4        |
|               | 4                       | 20.0       | 0.6        | 1.3        |
|               | 8                       | 18.8       | 0.7        | 1.4        |
| Wheat germ    | 0                       | 140        | 51.1       | 14.0       |
|               | 4                       | 136        | 50.8       | 14.0       |
|               | 8                       | 136        | 51.5       | 11.3       |

*UVB exposure conditions: oil layer thickness, 1.0 mm; UVB lamp distance, 10 cm.

Susceptibility of UVB-Exposed Oils to Thermal Treatment and Storage

To elucidate the stability of a 10-min UVB-exposed WGO, we analyzed the concentrations of vitamin D metabolites and tocopherols, the peroxide and acid values, and the organoleptic quality after thermal treatment and after storage of the oil samples.

The heating of UVB-exposed WGO at 100°C for 10 min resulted in a 50% increase of vitamin D2 (Δ = 521 ng/g) and a 66% increase in the vitamin D3 (Δ = 24.4 ng/g) concentration compared with the non-heated UVB-exposed WGO. In contrast, heating the oil at 180°C for 10 min resulted in a slight reduction of the vitamin D2 (Δ = −47.0 ng/g) and vitamin D3 (Δ = −2.8 ng/g) concentrations (Figure 3). Thermal treatment of the UVB-exposed and untreated WGO at 100°C had no effect on the analyzed markers for oxidation, while a thermal treatment at 180°C resulted in a slight reduction of the tocopherol concentrations and in a decrease in the peroxide value; the acid value remained unchanged (Table 2). Thermal treatment also affected the taste of the oil: the higher the treatment temperature, the lower was the organoleptic quality. The UVB exposure per se had only a small effect on the taste when the oil was heated at 180°C (Table 3).

The storage of UVB-exposed oil at room temperature also resulted in a rise of the vitamin D2 and the vitamin D3 concentrations (Figure 3). The highest vitamin D2 concentration was measured after the 2-week storage (Δ = 1157 ng/g; Figure 3A). The vitamin D3 concentration rose continuously during the 4-week storage and reached the highest values after...
4 weeks (Figure 3B). The tocopherol concentrations in the UVB-exposed oil remained slightly with the storage time; those of the untreated oil remained unchanged (Table 2). Both, the UVB-exposed and the -untreated oil showed increased peroxide values after the 4-week storage, no changes were observed for the acid values (Table 2). Organoleptic tests showed that the 4-week storage lead to deteriorated taste of the UVB-exposed and the -untreated oils, without showing any substantial difference between the UVB-exposed and the -untreated oil (Table 3). The aroma of the oils was not affected by the UVB exposure or the 4-week storage (Table 3).

**Efficacy of the UVB-Exposed Wheat Germ Oil to Improve the Vitamin D Status of Mice**

To evaluate the efficacy of UVB-exposed WGO to improve the vitamin D status, a feeding study with mice was conducted. The analyzed concentrations of vitamin D precursors and vitamin D in the WGO demonstrate that the applied UVB treatment was capable of increasing the vitamin D3 and vitamin D2 in this oil (Table 4). The tocopherol concentrations in the untreated and the UVB-exposed oils were comparable (Table 4). Mice of the three groups did not differ in their daily food intake (WGO: 3.03 ± 0.23 g, WGO-UV: 3.01 ± 0.14 g, and WGO-D3: 3.11 ± 0.07 g) and final body mass (WGO: 31.5 ± 3.0 g, WGO-UV: 31.8 ± 2.9 g, and WGO-D3: 32.1 ± 1.6 g).

Because any changes in 25(OH)D upon feeding vitamin D are usually becoming the higher the lower the vitamin D status is at baseline (27), all mice received a vitamin D-free diet 5 weeks prior to the treatment with the UVB-exposed or vitamin D3-supplemented WGO. The plasma concentrations of total 25(OH)D [25(OH)D2 + 25(OH)D3] after feeding the vitamin D-free basal diet for 5 weeks was below the LLOQ (n = 6). Feeding mice the diet with UVB-exposed oil (WGO-UV) or with vitamin D3 supplemented oil (WGO-D3) for 4 weeks resulted in markedly higher plasma concentrations of total 25(OH)D compared with feeding the untreated WGO-based diet without any vitamin D supplementation (WGO) (P < 0.001). However, the increase in the total plasma concentration of 25(OH)D was stronger in the WGO-D3 group than in the WGO-UV group (P < 0.001). The predominant form of the plasma 25(OH)D in the WGO-UV group was 25(OH)D2; the predominant form in the WGO-D3 group was 25(OH)D3 (Figure 4). The plasma concentration of ergosterol was below the LLOQ in all groups of mice (Table 5). All mice had comparable plasma concentrations of 7-DHC. Mice from the WGO and WGO-D3 groups had plasma concentrations of vitamin D3 that were below the LLOQ, whereas mice from the WGO-UV group had more than 10-fold higher LLOQ values (Table 5). By contrast, the WGO-D3 group showed a markedly higher plasma concentration of vitamin D3 than the WGO-UV group (P < 0.001); the plasma concentration of vitamin D3 in the WGO group was below the LLOQ. No differences between the three groups were observed in the plasma concentrations of α-tocopherol (Table 5).

Analysis of the D vitamer concentrations in the livers of the mice revealed no significant differences in the concentration of ergosterol (WGO: 12.0 ± 19.6 ng/g, WGO-UV: 3.92 ± 1.27 ng/g, and WGO-D3: 5.40 ± 1.27 ng/g) and 7-DHC (WGO: 94.3 ± 31.6 ng/g, WGO-UV: 84.7 ± 16.5 ng/g, and WGO-D3: 102 ± 46 ng/g). However, data showed distinct differences in the liver concentrations of vitamin D (Figure 5). Livers of mice from the WGO-UV group were characterized by extremely high vitamin D3 concentrations and high levels of 25(OH)D2, whereas the livers of mice from the WGO-D3 group had significantly higher vitamin D3 and 25(OH)D3 concentrations than those of mice from the two other groups (Figure 5).

**DISCUSSION**

The presented studies demonstrated that plant oils contain high amounts of ergosterol, but comparatively low amounts of 7-DHC. It was striking that the ergosterol concentrations in the plant oils were on average 100 times higher than the 7-DHC concentrations. It is assumed that plants are per se not capable of producing ergosterol or vitamin D2 (28), and that any of these metabolites are synthesized by endophytic fungi or by superficial fungal infections (13, 14, 29). Regarding 7-DHC, the analyses revealed 10 times higher concentration of this cholesterol precursor in the WGO than in the other oils. 7-DHC is an intermediate of the cholesterol synthesis pathway. It is well described that plants are capable of producing cholesterol (30, 31), which is assumed to be used for the synthesis of glycoalkaloids and ecdysteroids (32, 33). The 7-DHC has also been proposed to function as an UV light protector (34), because the 7-DHC absorbs UVB irradiation that would otherwise damage the ribonucleic acids. The detectable amounts of 7-DHC in the linseed, rapeseed, and pumpkinseed oil suggest that cholesterol is also synthesized in plants from the Linaceae, **TABLE 3 | Influence of thermal treatment and storage on taste and aroma of UVB-exposed and non-exposed wheat germ oil.**

| Exposure | Treatment | Taste | Points | Aroma | Points | Rank |
|----------|-----------|-------|--------|-------|--------|------|
| – – UVB | – UVB     | Not quite neutral rancid | 3 | Specific, neutral | 5 | 2 |
| – – WGO | – UVB     | Not quite neutral rancid | 3 | Specific, neutral | 5 | 3 |
| – – WGO | – WGO     | Not quite neutral rancid | 3 | Specific, neutral | 4 | 4 |
| – – WGO | – WGO     | Not quite neutral rancid | 3 | Specific, neutral | 5 | 6 |

*UVB exposure conditions: exposure time, 10 min; oil layer thickness, 1.6 mm; UVB lamp distance, 13 cm.

*The oil samples were tempered to 40°C.

*Correspondent: 1, poor; 2, insufficient; 3, satisfactory; 4, good; 5, excellent.

RT, room temperature.
The detection of vitamin D precursors in the plant oils prompted us to speculate that exposure of oils to UVB irradiation could convert ergosterol and 7-DHC into vitamin D$_2$ and vitamin D$_3$, respectively. Among the analyzed plant oils, the highest levels of vitamin D$_2$ and vitamin D$_3$ in response to an UVB irradiation were found in the WGO. After an 8-min exposure of thin-layered WGO, 1 g of this oil contained 1.5 μg vitamin D$_2$ and 0.08 μg vitamin D$_3$. We further found that the conversion rate of vitamin D precursors to vitamin D in the WGO was reduced by 40% if the oil layer thickness was increased from 1.0 to 3.2 mm. One gram of this thick-layered WGO provided in total a vitamin D content of 885 ng. With an average consumption of 12 g oil/day (38), a total of 10.6 μg vitamin D could be supplied by intake of UVB-exposed WGO, which matches 50% of the recommended daily vitamin D intake (1).

An interesting finding of this study was that the vitamin D content in the oils increased with the time of storage and a moderate thermal treatment. It is well described that the UVB photon converts the precursors, 7-DHC and ergosterol, to pre-vitamin D which in turn isomerizes to vitamin D by a thermal

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**TABLE 4** Characterization of the untreated and UVB-exposed wheat germ oils that were used in the mouse study.

| Wheat germ oil | Untreated | UVB-exposed$^a$ |
|----------------|-----------|-----------------|
| Ergosterol (μg/g) | 42.2      | 42.3            |
| 7-DHC (ng/g)   | 960       | 921             |
| Vitamin D$_2$ (ng/g) | <LLOQ   | 850             |
| Vitamin D$_3$ (ng/g) | <LLOQ   | 34.8            |
| Tocopherols (mg/g) |           |                 |
| α              | 1.32      | 1.34            |
| β              | 0.51      | 0.50            |
| γ              | 0.12      | 0.15            |

$^a$UVB exposure conditions: exposure time, 10 min; oil layer thickness, 3.2 mm; distance of the UVB-emitting lamp, 13 cm.

7-DHC, 7-dehydrocholesterol; LLOQ, lower limit of quantification (4.3 ng/g).
reaction (34, 39). Therefore, we assume that the preformed pre-vitamin D can convert to vitamin D in conditions with absent UVB irradiation. Our data further indicate that taste and aroma, and also biomarkers that are indicative of autoxidation such as the tocopherol concentration, peroxides, and free acids were not significantly influenced by a short-term exposure of the plant oils to UVB irradiation. This makes the short-term UVB treatment of plant oils to a safe and reliable technique to produce vitamin D supplements.

To evaluate the efficiency of UVB-exposed plant oils to improve the vitamin D status in vivo, we conducted a study with mice that were fed diets with either UVB-exposed WGO, untreated WGO, or WGO with supplemented vitamin D3. Here, we found that the UVB-exposed WGO is suitable to improve the vitamin D status of the mice as the group fed the UVB-exposed oil developed higher 25(OH)D plasma levels than the group fed the untreated oil. Compared with the group fed the vitamin D3-supplemented WGO, the UVB-exposed oil was less effective in increasing the 25(OH)D plasma concentrations. However, it should be noted that the livers of mice that received the UVB-exposed WGO stored huge amounts of vitamin D2 in comparison to that of mice fed the vitamin D3 supplemented oil. The increased storage of hepatic vitamin D2 in combination with the reduced plasma concentration of 25(OH)D2 in the group fed the UVB-exposed oil suggests that vitamin D2 is less appropriate as a substrate for hepatic hydroxylation than vitamin D3. It has been a debate for many years whether both forms of vitamin D are bioequivalent. A series of studies has shown that vitamin D2 does not increase 25(OH)D serum concentrations to the same amount as vitamin D3 does (40–42). The current data confirm the different efficacy of both vitamin D isoforms. However, we cannot exclude at this stage, that photo-isomers that are produced by the UVB treatment may also impact the bioavailability of the vitamin D form in UVB-exposed oil.

To conclude, plant oils that are commonly used in human nutrition contain considerable quantities of ergosterol, but small amounts of 7-DHC. Among the different analyzed oils, WGO has the highest amounts of vitamin D precursors. A short-term UVB irradiation was successful in increasing the vitamin D content of
FIGURE 5 | Liver concentrations of (A) vitamin D$_2$, (B) vitamin D$_3$, (C) total vitamin D (vitamin D$_2$ + vitamin D$_3$), (D) 25-hydroxyvitamin D$_2$, (E) 25-hydroxyvitamin D$_3$, and (F) total 25-hydroxyvitamin D (25-hydroxyvitamin D$_2$ + 25-hydroxyvitamin D$_3$) in mice fed diets with 10% of either wheat germ oil (WGO), UVB-exposed wheat germ oil (WGO-UV) or wheat germ oil that was supplemented with vitamin D$_3$ (WGO-D$_3$) for 4 weeks. Data represent means ± SD, n = 12. *Means not sharing a letter are significantly different (P < 0.05, Mann–Whitney U test for vitamin D$_2$, total vitamin D, 25-hydroxyvitamin D$_2$, and 25-hydroxyvitamin D$_3$; Games–Howell test for vitamin D$_3$; Tukey test for total 25-hydroxyvitamin D). #Values were below the lower limit of quantification (vitamin D$_2$, 5.0 ng/g; vitamin D$_3$, 10.5 ng/g; 25-hydroxyvitamin D$_2$, 0.3 ng/g; 25-hydroxyvitamin D$_3$, 2.1 ng/g).
the selected oils. The in vivo study has shown that UVB-exposed WGO can improve the vitamin D status, although less effective than vitamin D₃.

**AUTHOR CONTRIBUTIONS**

CB, BK, and GS conceived and designed the experiment. AB performed the experiment. AB and FH analyzed the data. AB, CB, and GS wrote the manuscript. BK and FH critically reviewed the manuscript.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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