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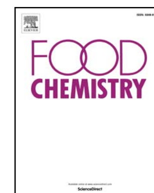

Stability of vitamin D_3 and vitamin D_2 in oil, fish and mushrooms after household cooking

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ABSTRACT

Information on the retention of vitamin D in food following household cooking is scarce. So far the retention of its metabolites vitamin D_3, vitamin D_2, and 25-hydroxyvitamin D_3 has shown that the type of food and the cooking method are the essential determinants, and there is no significant difference between the metabolites. We investigated the retention of vitamin D_3 and vitamin D_2 in sunflower oil, vitamin D_3 in rainbow trout, and vitamin D_2 in button mushrooms. The investigated cooking methods were boiling at different pH, steam cooking, microwave cooking, pan-frying, and oven baking.

There was no difference between the retention of vitamin D_3 and vitamin D_2 added to sunflower oil, which ranged from 70 to 99%. In rainbow trout, the retention of vitamin D_3 at 85–114% was not significantly different from 100%, except for panfrying at 85%. However, the retention of vitamin D_2 in mushrooms at 62–88% was significantly different from 100% ($p \leq 0.05$).

1. Introduction

Vitamin D is a group of fat-soluble sterols that is present in several forms. The two major forms, which differ in their side chain, are cholecalciferol (vitamin D_3), which is found mainly in foodstuffs of animal origin, and ergocalciferol (vitamin D_2), which is found in certain fungi such as wild mushrooms (Fig. 1).

Except from dietary intake, cholecalciferol can be synthesized in skin cells during exposure to sunlight (290–315 nm). Sunlight stimulates the conversion of 7-dehydrocholesterol (provitamin D_3), which naturally occurs in the body, to previtamin D_3, which thermally isomerizes into vitamin D_3 at body temperature (Wacker & Holick, 2013). Exposure to UV light also converts ergosterol (provitamin D_2), present in mushrooms or other fungi, to previtamin D_2, which then thermally isomerizes to vitamin D_2 (Keegan, Lu, Bogusz, Williams, & Holick, 2013). Ergocalciferol and cholecalciferol are biologically inert compounds; it is their metabolites that have biological activity. Vitamin D is converted, mainly in the liver, to 25-hydroxyvitamin D, which is further hydroxylated in the kidneys to the metabolically active form 1,25-dihydroxyvitamin D (Bikle, 2014). Individuals living at latitudes above 35°N or in air-polluted environments produce less vitamin D_3 because the winter sunlight lacks UVB and pollution reduces the amount of UVB that reaches the earth's surface. In these conditions, the human body is incapable of supporting the production of vitamin D_3 (Webb, 2006). The Institute of Medicine (IOM) recommends 50 nmol/L as a level of 25-hydroxyvitamin D that would be sufficient for skeletal health benefits for all persons (Institute of Medicine, 2011). Previous population-based studies from around the world have reported a prevalence of deficiency (< 50 nmol/L) to be approximately 25% of the population in Canada, 22–36% in the USA, 45–52% in New Zealand, 47–65% in Korea, and 31% in Australia (Daly et al., 2012), whereas it varies a lot in Europe, with yearly average prevalence of 40% for European countries (Cashman et al., 2016). Therefore, vitamin D deficiency represents a global public health problem and dietary intake has become increasingly important.

The recommended daily allowance (RDA) is 400–600 IU vitamin D, which is equivalent to 10–15 µg vitamin D/day (Institute of Medicine, 2011; Nordic Nutrient Recommendations, 2014). Vitamin D_3 and its metabolites are mostly present in foods of animal origin such as meat, offal, dairy products, eggs, and fish. Fish and fish products are regarded as the major dietary source of vitamin D_3, especially salmonids such as salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) (Jakobsen and Smith, 2017). Vitamin D_2 and its metabolites are present in high amounts in wild mushrooms, whereas their content in foods of animal origin is extremely low. Cultivated button mushrooms (*Agaricus bisporus*) exposed to UVB during production or after packaging have a high content of vitamin D2 (Keegan et al., 2013; Koyyalamudi, Jeong, Song, Cho, & Pang, 2009; Kristensen, Rosenqvist, & Jakobsen, 2012). The UVB exposure process has been approved in the European Union (EU) under the novel food Regulation (EC) No. 258/97 for production

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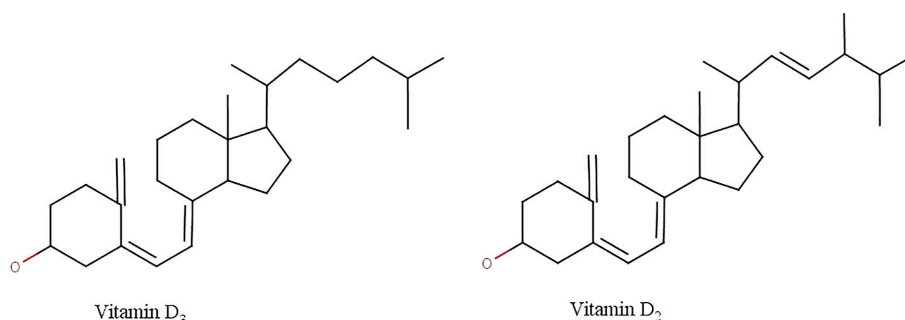

Fig. 1. Structure of cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2).

and sale of *Agaricus bisporus* with a content of vitamin $D_2 \leq 10\,\mu g/100\,g$ fresh weight in the EU-market (O'Mahony, 2016, 2017), which makes it possible for vitamin-D-enriched mushrooms to become one of the major dietary sources of vitamin D_2.

The information in food composition databases primarily represents the content of vitamin D in raw food, and the retention of vitamin D is usually estimated to be 100% in all foods (Pedersen et al., 2015; USDA, 2007, 2016). However, the investigation of bread, eggs, and margarine prepared by different cooking methods, in general, showed that retention was significantly different from 100%, but there was no difference in retention between the vitamin D metabolites vitamin D_3, vitamin D_2, and 25-hydroxyvitamin D_3 (Jakobsen & Knuthsen, 2014).

We investigated the retention of vitamin D metabolites in food during cooking methods usually performed in households, such as boiling, frying, and baking, as well as whether the addition of lemon juice affected the retention of vitamin D. Sunflower oil was used as a matrix in which the recovery of vitamin D_3 and vitamin D_2 was tested concurrently in the same food matrix, and farmed rainbow trout (*Oncorhynchus mykiss*) and UVB-exposed button mushrooms (*Agaricus bisporus*) represented dietary sources of vitamin D_3 and vitamin D_2, respectively.

2. Materials and methods

2.1. Samples

2.1.1. Sunflower oil

A mixed standard of cholecalciferol (Cholecalciferol C9774, Sigma-Aldrich, Denmark A/S) and ergocalciferol (Ergocalciferol E1007, Sigma-Aldrich, Denmark A/S) was prepared in *n*-heptane (LiChrosolv 104390, Merck Millipore, Germany) to a concentration of 2 µg/mL of each vitamer. To prepare each sample, 5 g of sunflower oil (Ollineo, Netto, Denmark) was spiked with 375 µL of the standard mixture, followed by vortexing, to achieve a concentration of 150 ng vitamer/g of sunflower oil. In total, 8 groups (3 samples per group) were prepared. One group of samples was untreated and served as a control of the vitamin D content. The other groups were treated by different cooking methods, as shown in Table 1. The table with the experimental design is shown in the Supplementary online material (SOM, Section S1, Table S1).

2.1.2. Rainbow trout

Four farmed rainbow trout fish (Musholm A/S, Reersø, Denmark) within the weight range of 1.5–2.9 kg were used. From each of the fish, 6 steaks were prepared. Each steak was separated along the spine (right and left looking from the head), and the skin and bones were removed. The weight of each of the fish fillets was approximately 100 g. Fillets from the left half were used for the analysis of the raw parts, whereas the counterpart on the right half was heat treated. Photos of the setup and a more detailed description can be found in the Supplementary online material (SOM, Section S2, Fig. S2).

Table 1
Specification of cooking procedures.

Food item	Treatment	Time (min)	Temperature (°C)
Sunflower oil fortified with vitamin D_3 and vitamin D_2	Air	180	20 ± 1
	Oven (Low temperature, Long time)	30	110 ± 10
	Oven (High temperature, Short time)	10	210 ± 10
	Pan-frying, high level	1	n.m.
	Microwave, high power	1	n.m.
	Lemon juice (pH 4.5)	10	20 ± 1
	Lemon juice (pH 2.5)	10	20 ± 1
Rainbow trout	Boiling	8	90 ± 2
	Boiling, lemon juice (pH 4.0)	8	90 ± 2
	Steam cooked	6	87 ± 3
	Microwave cooked	2	n.m.
	Pan-fried	8	n.m.
	Baked, oven (uncovered)	30	110 ± 9
	Baked, oven (uncovered)	10	210 ± 12
	Baked, oven (covered)	15	190 ± 14
Mushrooms bio-fortified with vitamin D_2	Boiling	20	90 ± 2
	Boiling, lemon juice (pH 3.5)	20	90 ± 2
	Pan-fried, high heat	5	n.m.
	Pan-fried, low heat	20	n.m.
	Baked, oven (uncovered)	90	70 ± 4
	Baked, oven (uncovered)	20	200 ± 9

n.m. = *not measured.*

2.1.3. Mushrooms

24 packages of 250 g fresh button mushrooms (*Agaricus bisporus*) each (Egehøj Champignon A/S, Veflinge, Denmark), were transported to National Food Institute, Technical University of Denmark and immediately exposed to a UVB-dose similar to 2 SED from a UVB-lamp specifically developed for optimal vitamin D production in dairy cows (Jakobsen et al., 2015). The irradiation on the mushrooms was measured at 312 nm using a handheld ILT 1400-BL photometer equipped with a SEL005/TLS312/TD detector (International Light Technologies, Peabody, MA). The irradiance varied from 840 to 1200 $\mu W/cm^2$ (see SOM, Section S3, Fig. S3). The UVB-exposure was performed in four batches with 6 packages in each batch. After exposure, the mushrooms were cleaned and the stalk was cut off. One batch (n = 6) was used to analyze the content of vitamin D_2 in raw mushrooms, while the samples for the heat treatment (n = 3) were randomly chosen from the other 3 batches (see SOM, Section S3, Fig. S3). The portions for each individual heat treatment were approximately 250 g mushrooms.

2.2. Heat treatment

The heat treatments are listed in Table 1. All of the heat treatments were performed without adding any other ingredient. The fish were treated in an oven with and without aluminum foil wrapped around the trout fillet, imitating the procedure often performed in households. Freshly squeezed lemon juice was used to adjust the pH in sunflower oil, trout, and mushrooms. To each portion of sunflower oil, 2 drops of lemon juice were added to obtain a pH of 4.5, whereas 5 drops of lemon juice were added to obtain a pH of 2.5. To each portion of boiled trout and mushrooms, lemon juice was added until the pH reached 4.0 in the water that was used for the trout and 3.5 in the water that was used to boil the mushrooms.

2.3. Weighing and homogenization

All of the samples were weighed raw and 30 min after heat treatment. All of the raw and processed samples were homogenized in a kitchen blender (FP220, Kenwood, United Kingdom) for 20 s and weighed in plastic bags, and the oxygen was replaced by nitrogen before closing the bags. The samples were stored at maximum of −20 °C until analysis, which took place within one month.

2.4. Design

Each heat treatment was performed three times independently of each other; e.g., three samples of steam-cooked fish were heated at three different times, whereas the analytical results represent a single determination of vitamin D metabolites.

2.5. Cooking facilities

The samples were baked in a Siemens stove (HL T650, Germany), cooked on a Bosch induction cooktop (PIE775N14E, Germany), and heated in a Panasonic microwave stove (NN E252W, Japan, 1200 W). Pan-frying was conducted in a pan (Aldente Palermo, Teflon Platinum coating, diameter = 180 mm). Furthermore, ordinary household cooking equipment, such as pots, baking trays, and sieves, were used for different kinds of heat treatments.

2.6. Temperature and pH measurements

The temperature in the oven during heating and the temperature in the middle of the samples were measured by a digital thermometer (Testo 735-2, Testo AG, Lenzkirch, Germany), whereas the final temperatures were measured by an analogue thermometer. The pH of the solution with added lemon juice was measured with a pH meter (PHM220, Radiometer analytical, France).

2.7. Analytical method

2.7.1. Vitamin D

For the quantification of vitamin D_3 and vitamin D_2 in sunflower oil, a previously described LC-ESI-MS/MS method for vitamin D_3 and 25-hydroxyvitamin D_3 in pork (Burild, Frandsen, & Jakobsen, 2014; Burild, Frandsen, Poulsen, & Jakobsen, 2014) was modified to include quantification of vitamin D_2. Standards of vitamin D_3 and vitamin D_2 were obtained from Sigma-Aldrich (Steinheim, Germany), whereas the deuterated internal standard 26,26,26,27,27,27-*d*6-vitamin D_3 (*d*6-D_3), was from Chemaphor Inc. (Ottawa, Canada). Prior to the extraction of 1 g oil, the deuterated internal standard listed above was added to the samples for analyses of vitamin D_3 and vitamin D_2. In short, sunflower-oil samples were saponified with ethanolic potassium hydroxide overnight at room temperature. The unsaponifiable matter was extracted by liquid/liquid extraction, cleaned-up by a silica solid-phase extraction (SPE), and derivatized with 4-phenyl-1,2,4-triazole-3,5-dione (PTAD; Sigma-Aldrich, Steinheim, Germany) prior to the identification and quantification of vitamin D_3 and vitamin D_2 by a triple quadrupole MS detector. The equipment was from Agilent (LC 1200 and MS 6470 series Triple Quad MS, Agilent Technologies, Santa Clara, CA).

For analyses of fish and mushrooms, a HPLC-UV/PDA method was applied (Jakobsen, Maribo, Bysted, Sommer, & Hels, 2007; Kristensen et al., 2012). In short, vitamin D_2 and vitamin D_3 were added as internal standards to 1 g of fish and 10 g of mushrooms, respectively. The saponification, the liquid-liquid extraction, and the SPE were similar as for sunflower oil. Furthermore, a normal-phase semi-preparative HPLC steps were included before the final separation, detection, and quantification, which was done by reverse-phase chromatography coupled to a photo-diode array detector (Alliance 2695; UV-detector 2487; PDA-detector 2996, Waters, Milford, MA).

The accuracy of the methods was secured by obtaining approved results i.e. within limits for the certified reference materials (Milk powder, CRM421, IRMM, Geel, Belgium) and a z-score < 1 for proficiency testing materials (Milk Powder, FAPAS 2184, FAPAS, York, United Kingdom). Furthermore, the recovery of added D_3 was 99.4 ± 6.6% (n = 3) and 97.2 ± 4.3% (n = 3) for UV/DAD and LC-MS/MS, respectively, and added D_2 at 99.7 ± 2.9% (n = 3) and 98.7 ± 6.2% (n = 10) for UV/DAD and LC-MS/MS, respectively. The consistency and precision of the methods during the studies were checked by analysis of control-salmon and control-mushrooms house-reference materials. In control-salmon, 15.4 ± 0.2 µg of vitamin D_3/100 g was determined using the HPLC-UV/DAD method (n = 8), which was similar to the value obtained by the LC-MS/MS method (n = 18) (15.2 ± 0.8 µg vitamin D_3/100 g). For the control-mushroom, the content of vitamin D_2 determined by HPLC-UV/DAD and LC-MS/MS was 16.8 ± 1.1 µg vitamin D_2/100 g (n = 10) and 17.0 ± 1.0 µg vitamin D_2/100 g (n = 5), respectively. All of the analyses were conducted in a laboratory that was accredited to perform the analyses according to ISO17025.

2.7.2. Weight loss and true retention

The samples were weighed before and after heat treatment to calculate the percentage of weight loss; see Eq. (1). Furthermore, weight data were combined with the amount of vitamin D in the raw samples and the amount of vitamin D in the heat-treated samples to calculate the true retention. The true retention calculation is exemplified by vitamin D_3 in Eq. (2) (Murphy, Criner, & Gray, 1975).

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

$$\%True\ retention = \left(\frac{\mu g\ vit\ D3\ per\ 100\ g\ of\ cooked\ food * amount\ of\ cooked\ food}{\mu g\ vit\ D3\ per\ 100\ g\ of\ raw\ food * amount\ of\ raw\ food}\right) * 100 \quad (2)$$

2.7.3. Statistical analysis

The data for the retention of vitamin D in sunflower oil, fish, and mushrooms were statistically evaluated using JMP® Statistical Discovery software version 13.0 (SAS Institute Inc. Cary, NC, USA). A *t*-test was used to evaluate if the retention differed from 100%, whereas a one-way ANOVA was used to test the retention of vitamin D in food after different cooking methods. The Tukey-Kramer test was used to examine the differences between the heating procedures and to compare different cooking methods. A p-value ≤ 0.05 was classified as a significant difference. All of the results are given as mean ± SD.

Table 2
Content of vitamin D metabolites in raw samples.

µg/100 g raw sample	Vitamin D_3	Vitamin D_2	Sample number (n)
Sunflower oil fortified with vitamin D_3 and vitamin D_2	14.5 ± 0.6	11.2 ± 0.6	3
Rainbow trout	7.5 ± 1.7	< 0.1	24
Mushrooms bio-fortified with vitamin D_2	< 0.01	19.2 ± 2.4	6

Table 3
Sunflower oil – true retention of vitamin D_3 and vitamin D_2.

	Heat treatment		Spiked sunflower oil	
	Temp, (°C)	Time, (min)	% True retention	
			Vitamin D_3	Vitamin D_2
Air	20 ± 1	180	96 ± 6^{ab}	97 ± 4^{a}
Oven (Low temperature, Long time)	110 ± 10	30	85 ± 7^{bc*}	89 ± 5^{ab*}
Oven (High temperature, Short time)	210 ± 10	10	76 ± 2^{cd*}	79 ± 3^{bc*}
Pan-frying, high level	n.m.	1	70 ± 7^{d*}	72 ± 5^{c*}
Microwave, high power	n.m.	1	84 ± 3^{bcd*}	88 ± 3^{ab*}
Lemon juice (pH 4.5)	20 ± 1	10	97 ± 3^{ab}	99 ± 4^{a}
Lemon juice (pH 2.5)	20 ± 1	10	99 ± 3^{a}	99 ± 4^{a}

Number of repetitions for each treatment: n = 3. The different letters in a column indicate significant differences ($p \leq 0.05$). A star (*) indicates a retention significantly different from 100% ($p \leq 0.05$). n.m. = *not measured.*

3. Results

The vitamin D contents found in the raw samples of sunflower oil, fish, and mushrooms are shown in Table 2. The true retention of vitamin D in fortified sunflower oil following each of the seven different heat treatments is shown in Table 3.

In rainbow trout, the vitamin D_3 content found in the raw samples was 7.5 ± 1.7 µg/100 g, whereas the weight loss ranged from 13 to 19%. The final temperature in the inner parts of the cooked fish fillets was measured to be 69 ± 3 °C. The temperature, time, and true retention results are shown in Table 4.

The vitamin D_2 content was 19.1 ± 2.4 µg/100 g raw mushrooms. The weight loss ranged from 24 to 49%. The temperature, time, true retention, and weight loss are shown in Table 5.

4. Discussion

There are limited dietary sources of vitamin D and information on its stability during processing and storage is scarce. The stability of vitamins depends on various conditions such as temperature, oxygen, light, moisture, pH, and duration of heat treatment; this information is limited in the case of vitamin D metabolites (Lešková et al., 2006). Essential for retention studies is the application of a validated analytical method with an appropriate accuracy and precision, which includes the use of internal standard in vitamin D method. In our study the methods were run with an inter-day precision < 7%. Furthermore, in our design we included appropriate control of the temperature used. Upreti, Mistry, & Warthesen (2002) examined the stability of vitamin D_3 in fortified cheese stored at room temperature and in a refrigerator (4–6 °C) and reported no losses during 9 months of storage. Furthermore, Kaushik, Sachdeva, & Arora (2014) found statistically insignificant losses of vitamin D_2 in fortified milk during pasteurization, boiling, and sterilization. However, the retention of vitamin D in different foods has been investigated and several contradictory results have been reported; the studies have shown that the retention of vitamin D varies between different foodstuffs and cooking methods. Bhuiyan et al. (1993) reported very modest loss of vitamin D in smoked Atlantic mackerel. Mattila, Ronkainen, Lehikoinen, and Piironen (1999) investigated the vitamin D retention in fish, eggs, and wild mushrooms following cooking and storage. They reported relatively high vitamin D_3 retention in baked fish (78–104%) and vitamin D_2 retention in pan-fried wild mushrooms (80–100%).

Table 4
Rainbow trout (*Oncorhynchus mykiss*) – true retention of vitamin D_3 and weight loss of cooked samples.

	Heat treatment			Fish (Rainbow trout)	
	Temp, (°C)	Time, (min)	Final T (°C)	True retention % Vitamin D_3	Weight loss %
Boiling	90 ± 2	8	68 ± 2	87 ± 16^{ab}	14 ± 1.3^{ab}
Boiling, lemon juice	90 ± 2	8	68 ± 3	102 ± 9^{ab}	14 ± 1.1^{ab}
Steam cooked	87 ± 3	6	67 ± 2	97 ± 5^{ab}	13 ± 2.6^{b}
Microwave cooked	n.m.	2	69 ± 3	101 ± 1^{ab}	19 ± 0.8^{a}
Pan-fried	n.m.	8	65 ± 1	85 ± 6^{b*}	18 ± 2.9^{ab}
Baked, oven (uncovered)	110 ± 9	30	70 ± 2	114 ± 13^{a}	15 ± 0.6^{ab}
Baked, oven (uncovered)	210 ± 12	10	65 ± 2	87 ± 14^{ab}	17 ± 2.0^{ab}
Baked, oven (covered)	190 ± 14	15	80 ± 2	93 ± 8^{ab}	16 ± 1.6^{ab}

Number of repetitions for each treatment: n = 3, i.e., for each cooking method 6 samples (3 raw and 3 cooked). The different letters in a column indicate significant differences ($p \leq 0.05$). A star (*) indicates a retention significantly different from 100% ($p \leq 0.05$). n.m. = *not measured*

Table 5
Mushrooms – true retention of vitamin D_2 and weight loss.

	Heat treatment		Vitamin D_2 bio-fortified mushrooms		
	Temp, (°C)	Time, (min)	Final T (°C)	True retention % Vitamin D_2	Weight loss %
Boiling	90 ± 2	20	n.m.	62 ± 14^{b}	26 ± 0.3^{a}
Boiling, lemon juice	90 ± 2	20	n.m.	80 ± 5^{ab}	24 ± 0.3^{a}
Pan-fried, high heat	n.m.	5	n.m.	81 ± 1^{ab*}	27 ± 0.2^{b}
Pan-fried, low heat	n.m.	20	n.m.	88 ± 9^{a}	35 ± 0.6^{c}
Baked, oven (uncovered)	70 ± 4	90	n.m.	74 ± 2^{ab*}	49 ± 0.9^{d}
Baked, oven (uncovered)	200 ± 9	10	n.m.	67 ± 3^{b*}	48 ± 2^{d}

Each cooking method was repeated 3 times, except for "Boiling" n = 2. Six raw samples were analyzed. The different letters in a column indicate significant differences ($p \leq 0.05$). A star (*) indicates a retention significantly different from 100% ($p \leq 0.05$). n.m. = *not measured.*

We found no differences between the retention of vitamin D_3 and vitamin D_2 in spiked sunflower oil during various heat treatments. Although there was no significant difference, vitamin D_2 tended to have a slightly higher retention than vitamin D_3. A similar trend has been previously observed during the processing of pure substances of vitamin D_3 and vitamin D_2 (Grady & Thakker, 1980) and vitamin D_3 and vitamin D_2 added to bread (Jakobsen & Knuthsen, 2014). Furthermore, the highest baking temperature resulted in the lowest retention (Jakobsen & Knuthsen, 2014), which was also the case in vitamin-D-spiked sunflower oil.

The retention of vitamin D3 in fish was not significantly different from 100%, except when pan-frying was used as a cooking method. The

retention ranged from 85 to 114%, which was in accordance with the observation by Mattila et al. (1999), who did not find a significantly different loss from 100% for fish covered with foil and treated for 20 min in an oven at 172–200 °C. Oven-cooking at a lower temperature, such as 110 °C for 30 min, resulted in no loss of vitamin D_3, even though the final temperature in the fish was similar to fish cooked at 210 °C. Covering the fish with foil did not result in any difference in retention from fish treated in an oven without foil.

The retention of heat-treated mushrooms was 62–88%, for which pan-frying on a strong fire, and baking in the oven at both low temperature (70 °C) and high temperature (200 °C) were significantly different from 100%. The retention of vitamin D_2 in mushrooms was significantly lower for samples that were boiled in water or baked in the oven (200 °C), which showed retention of 62–67%, compared with the highest retention of 88% for mushrooms pan-fried at a low temperature. Mattila et al. (1999) investigated pan-frying of two types of wild mushrooms for 5 min and found retention of 80–82% for one species (*C. Cibarius*) and 97–100% for another species (*C. Tubaeformis*). In our investigation, the vitamin D_2 bio-fortified button mushrooms that were pan-fried for 5 min at a high temperature showed a similar retention as *C. Cibarius*, i.e., 81 ± 1%.

The heat treatment of the mushrooms samples was generally longer than the heat treatment of the fish samples, which could cause a lower retention of vitamin D. The duration of the heating procedures performed on the fish samples ranged from 2 min in microwave cooking to 30 min in the oven, whereas the mushrooms samples were heated for longer: 5 min of pan-frying to 90 min in the oven in order to simulate normal cooking procedure. As fish are very often a source of unsafe food consumption caused by fast growth of bacteria, the final temperature in the inner parts of the fish was measured to ensure that the final temperature reached 69 ± 3 °C. This temperature is high enough for safe consumption according to Davidson and Jaine (2014), who reported that fish is cooked when its innermost parts have reached a temperature of approximately 63 °C.

In one of our previous studies, the retention of vitamin D in rye bread was determined to be lower than in white bread (Jakobsen & Knuthsen, 2014). The investigation of the effect of pH between 4.0 and 7.1 in rye bread dough adjusted by adding hydrochloric acid showed that acidic environment did not significantly affect the retention of 71–75% (Jakobsen, unpublished). In the current study, we investigated the effect of different pH adjusted by adding lemon juice in sunflower oil at room temperature, and in oven-treated fish and boiled mushrooms. An acidic environment at room temperature had an insignificant effect on the vitamin D retention in sunflower oil. The boiling of fish and mushrooms samples resulted in the lowest retention rates out of all the cooking methods normally used in households. Boiling in an acidic environment, pH 4.0 for fish and pH 3.5 for mushrooms resulted in a higher retention than boiling in neutral water. In both types of samples, the vitamin D retention of samples that were cooked in a neutral environment was approximately 20% lower than the vitamin D retention in samples cooked in an acidic environment. Thus, the addition of lemon juice preserved vitamin D in fish and mushrooms during cooking, which could be owing to the antioxidant properties of ascorbic acid that was present in the acidic solution (Bendich, Machlin, Scandurra, Burton, & Wayner, 1986; Hajimahmoodi et al., 2012). These results indicate that the retention of vitamin D is improved by adding lemon juice prior to the heat treatment.

To our knowledge, this is the first study that examined the retention of the two main vitamin D metabolites spiked in a food based medium such as sunflower oil, and tested the effect of lemon juice on the retention of vitamin D during the household cooking. We used a highly accurate LC-MS/MS method. Furthermore, the experimental design took into consideration the differences in the distribution of the vitamin D in the fish. Analyzing the counterpart of the processed fish fillet enabled more reliable examination of the retention, since the assumption was that the content of the vitamin D is the same in the both parts separated by the spine.

A limitation of our study is the omission of the investigation of 25-hydroxyvitamin D_3. However, the content of 25-hydroxyvitamin D_3 in the trout was shown to be 0.09 μg/100 g in another study (Jakobsen & Smith, 2017). This content was close to the limit of quantification for the method, which we do not find appropriate for a retention study. Furthermore, Jakobsen and Knuthsen (2014) examined the retention of vitamin D_3 and 25-hydroxyvitamin D_3 during three different cooking methods in eggs and no difference in retention was found.

The results obtained for fish indicate that no correction of vitamin D should be included for the calculation of dietary intake. In contrast, the vitamin D retention in household-cooked bio-fortified mushrooms should be regarded as different from 100%. Even though we found a significant difference between the cooking methods, an average retention of 75% should be used to calculate the dietary intake. Mushroom soups prepared from vitamin D_2 enriched mushrooms has been shown to be an excellent source of vitamin D_2 which had equivalent bioavailability as a vitamin D_2 supplement (Urbain, Singler, Ihorst, Biesalski, & Bertz, 2011). A portion of soup prepared from 100 g of vitamin D enriched mushroom with a content of 10 μg/100 g (legal for the EU-market) would provide 50–75% of the recommended daily intake, while a portion size of 200 g of fish would provide around 100% of the recommended daily intake. In summary, the dietary intake of vitamin D plays an enormous role in the prevention of its deficiency in humans, and meals composed of fish and vitamin D enriched mushrooms are good sources to ensure the recommended daily intake.

5. Conclusion

The retention of vitamin D in sunflower oil, fish, and mushrooms was determined after household cooking. No significant difference was found between the retention of vitamin-D_3- and vitamin-D_2-spiked sunflower oil. The true retention of vitamin D_3 in fish was not significantly different from 100%, except after pan-frying, which showed a true retention of 85%. In mushrooms, the retention of vitamin D_2 was significantly lower than 100% with retention rates ranging from 62 to 88%. In fish and mushrooms, the same trend was observed: the use of higher temperatures had a negative effect on vitamin D retention, whereas a lower acidity caused by the addition of lemon juice increased the retention of vitamin D.

Cooking may cause a significant loss of vitamin D, but the degree of loss depends on the combination of foodstuffs and the heating process. Based on the results in this study, we recommend that the retention of vitamin D_3 in fish and vitamin D_2 in bio-fortified mushrooms should be estimated at 100% and 75%, respectively.

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JJ designed the study in mushrooms, and JJ and PL designed the study in sunflower oil and fish. PL conducted the sampling and analyses of sunflower oil and fish samples. The authors are grateful for the assistance from Martin Lund-Larsen in conducting the study in mushrooms, and from Heidi Jahn for skillful technical assistance with the analyses of vitamin D. PL made the data analysis and wrote the manuscript, JJ reviewed and approved the final version of the manuscript. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. None of the authors had personal or financial conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2018.01.182.

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