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Analytical Methods

Stability of vitamin D in foodstuffs during cooking

Jette Jakobsen *, Pia Knuthsen

Division of Food Chemistry, National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, DK-2860 Søborg, Denmark

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ABSTRACT

We investigated the retention of vitamin D3 and 25-hydroxyvitamin D3 in eggs, vitamin D3 in margarine, and vitamin D3 and vitamin D2 in bread. Our set-up illustrated the cooking methods usually performed in households i.e. boiling, frying in pan and oven, and baking. All experiments were performed three times independently of one another. The retention of vitamin D compounds in eggs and margarine during heat treatment in an oven for 40 min at normal cooking temperature showed retention at 39–45%, while frying resulted in retention at 82–84%. Boiled eggs were found to have a similar level of retention (86–88%). For bread baked, as recommended in the recipe, the retention of vitamin D3 in rye bread at 69% was lower than the retention in wheat bread at 85%. A similar observation was made for vitamin D2, although the retention was slightly higher, 73% and 89%. No difference between retention of vitamin D3 and 25-hydroxyvitamin D3 in eggs was shown. Cooking may cause detrimental loss of vitamin D, but it depends on the actual foodstuffs and the heating process. Further research is needed to optimise cooking procedures to enhance retention of vitamin D. Vitamin D retention should be taken into account in future calculations of dietary intake of vitamin D.

1. Introduction

Vitamin D deficiency is a great health concern for both children and adults. The vitamin D deficiency diseases are rickets in children and osteomalacia in the elderly. Inadequate vitamin D status increases a risk for fractures in elderly people, while an association between vitamin D status and risk for cancers, cardiovascular diseases, diabetes, and inadequate immune defense is suspected but lack to be proven as a cause or relationship (Holick, 2007; IOM, 2011).

Vitamin D is a fat-soluble vitamin that humans produce in skin when exposed to UV light (290–315 nm), and obtain through dietary intake. There are several high risk groups of vitamin D deficiency: individuals who avoid sun exposure, people with dark-pigmented skin, older people, those in residential care or nursing homes. Dietary intake is essential all year round for people in high risk groups and during winter for people living at latitudes above 35° (Holick, 2007; IOM, 2011). A recent review on dietary intake of vitamin D in adults and children shows that a dietary intake of vitamin D in general is inadequate compared to recommendations (Kiely & Black, 2012).

The vitamin D content in foods are composed of the parent vitamin D, cholecalciferol ($vitD_3$) and ergocalciferol ($vitD_2$), and their metabolites, the hydroxylated and dihydroxylated vitamers mainly 25-hydroxyvitamin D (25OHD). $VitD_3$ and its metabolites are only present in foods of animal origin, fish, meat, eggs, and milk (Foote et al., 2004; Jakobsen & Saxholt, 2009; Mattila, Piironen, Uusirauva, & Koivistoinen, 1995). $VitD_2$ is present in wild mushrooms in high amount, while foods of animal origin may contain low amounts of $vitD_2$ and its metabolites (Jakobsen & Saxholt, 2009; Mattila, Piironen, Uusirauva, & Koivistoinen, 1994; Teichmann, Dutta, Staffas, & Jagerstad, 2007).

The stability of vitD in fortification regiments has been tested during spray-drying of milk and production of vitD fortified UHT-milk and cheese; the loss of vitD was found to be insignificant in these products (Hanson & Metzger, 2010; Indyk, Littlejohn, & Woollard, 1996; Wagner et al., 2008).

An investigation of the stability of vitD in pure and dry form showed that heating to 150 °C in the presence of air caused almost total destruction (Chen, Raymond Terepka, Lane, & Marsh, 1965). A controlled study of $vitD_3$ at temperatures 100–170 °C, showed a temperature- and time-dependent irreversible transformation to pyrocholecalciferol and isopyrocholecalciferol (Pelc & Marshall, 1978). In an acidic environment vitD might be isomerised to isotachysterol (Agarwal, 1990; Jin, Yang, Yang, Liu, & Zhang, 2004; Seamark, Trafford, & Makin, 1980).

A calculation of dietary intake of vitD is generally based on information of contents of vitD in foods and intake of foodstuffs. The information in food composition databases is mainly the content in raw foods. The dietary intake of processed foods needs to be corrected by the retention during the cooking process. In a review covering retention during heat treatment it is emphasized that the current information on the fate of vitD metabolites during heating

* Corresponding author. Tel.: +45 3588 7415.
E-mail address: jeja@food.dtu.dk (J. Jakobsen).

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and processing is incomplete (Leskova et al., 2006). As information on retention is incomplete, it is current practice not to correct or to use the retention 100% for information in food composition data bases and in calculation of dietary intake of vitamin D (Pedersen et al., 2010; USDA, 2007).

A recent study on fortification of bread with $vitD_3$ has identified lower retention than found previously (Madsen et al., 2013; Natri et al., 2006).

In this study we investigated the retention of vitD during cooking methods usually performed in households i.e. frying, baking and boiling. We included retention of $vitD_3$ and $25OHD_3$ in eggs, $vitD_3$ in margarine, and $vitD_3$ and $vitD_2$ in various breads.

2. Materials and methods

2.1. Food samples

2.1.1. Eggs

In total 18 eggs (>63 g, LIVSKRAFT, Hedegaard, Denmark) were used for the retention studies in eggs. No other ingredient than the eggs was included in these heat treatments. Each of the three cooking procedures were repeated three times. For each of the individual treatment 2 eggs were heated. The cooking procedures were as follows: the boiled eggs were cooked for 10 min in boiling water, the scrambled eggs were initially stirred for 30 s and heated for 3 min in a pan (Aldente Palermo, Teflon Platinum coating, diameter = 180 mm), the oven-heated eggs were initially whipped with a whisk for 2 min followed by heating in an oven proof dish (diameter = 135 mm) in the oven for 40 min which had been set at 175 °C. Furthermore, 6 eggs (>63 g, Brunch æg, Hedegaard, Denmark) were used for the retention studies in cakes (see Section 2.1.3).

2.1.2. Margarine

Hard margarine (500 g package x-tra, Mattfett, Smørbart plantefedtstof, Margarin, 70% fat, Coop, Denmark), labelled 10 μg vitD/100 g was used for testing the retention by heating margarine on its own. Margarine in portions of 40 g was fried in a pan (same as in Section 2.1.1) for 3 min, and in the oven proof dish (same as in Section 2.1.1) heated in the oven for 40 min which had been set at 175 °C. Furthermore, the margarine was used as an ingredient in cakes (see Section 2.1.3).

2.1.3. Cakes

The ingredients for each cake were: 375 g flour, 4.5 g baking powder, 150 g margarine (see Section 2.1.2), 330 g sugar, 2 eggs (see Section 2.1.1) and 310 g milk (0.5% fat).

A subsample of cake mixture was taken for vitD analysis before the remaining cake mixture was put into a baking tin with Teflon coating (size 110 * 300 * 70 mm), and baked in the oven for 60 min which had been set at 175 °C.

2.1.4. Dry yeast

Dry yeast (Malteserkors, Lallemand, Canada) in combination with

$VitD_3$ trials: Concentrated $vitD_3$ (Oily Vitamin Blend 02–185, BASF, Germany), labelled content 2500–2750 μg $vitD_3$/g. The analytical result confirmed a content of 2500 ± 100 μg $vitD_3$/g.

$VitD_2$ trials: $VitD_2$ enriched dry yeast (Lalmin, VitaD, Lallemand Inc., Canada). Expected content approximately 627.5 μg $vitD_2$/g, the analytical result showed 850 ± 34 μg $vitD_2$/g.

2.1.5. Bread

Wheat bread: Six 1 kg ready-to-bake flour (Amo Youghurtbrød, LantmännenCerealia, Vejle, Denmark) were used to produce 12 loaves of bread. Six were enriched with vitamin D_2 and six were enriched with vitamin D_3.

The dough for two loaves of bread was made from 1 kg ready-to-bake flour, 12 g dry yeast (see Section 2.1.4), approx. 600 g tap water, and approx. 200 mg $vitD_2$ enriched yeast or 72 mg $vitD_3$ oil (see Section 2.1.4). The bread was left to rise for 30 min. Before weighing the dough, a sub-sample (30 g) was taken for analysis of vitD in the dough. From the remaining dough (approx. 1500 g), two loaves of bread were made, each loaf was put into a baking tin (same as in Section 2.1.3), and baked in an oven. Each of the six pairs of bread were baked in the same oven at the same time which had been set at 200 °C, one loaf was baked for 30 min (recommended in the recipe) and the other loaf was baked for 60 min.

Rye bread: Six 1 kg ready-to-bake flour (Amo Mørkt rugbrød, LantmännenCerealia, Vejle, Denmark) were used to produce six loaves of bread. Three were enriched with vitamin D_2 and three were enriched with vitamin D_3.

The dough for one loaf of bread was made from 1 kg ready-to-bake flour, 6 g dry yeast (see Section 2.1.4), approx. 900 g tap-water, and approx. 200 mg $vitD_2$ enriched yeast or 72 mg $vitD_3$ oil (see Section 2.1.4). The bread was left to rise for 30 min. Before weighing the dough, a sub-sample (30 g) was taken for analysis of vitD in the dough. From the remaining dough (approx. 1800 g), one loaf of bread was made and put into a baking tin (EVA TRIO, anti-stick coating, size 110 * 300 * 100 mm). One loaf enriched with $vitD_2$ yeast and one loaf enriched with $vitD_3$ oil were baked together in an oven which had been set at 200 °C for 60 min as recommended in the recipe.

2.2. Cooking facilities

For practical reason three different stoves were used during the studies. Margarine and eggs were heated in a Siemens stove (HL T650, Germany), cakes were baked in a stove manufactured by Gorenje (HEC789W, Slovenia), and bread in a stove from Italy (ILVE PL-90-MP). Furthermore, ordinary household cooking equipments, like pans and pots, were used for frying and cooking.

2.3. Temperature measurements

Three different temperature regiments were used during the studies. For eggs and margarine a normal thermometer was used to measure the final temperatures. Furthermore the temperature in the oven during the heating of eggs and margarine was measured by Testo 735-2 (Testo AG, Lenzkirch, Germany). The temperature in the oven during the heating of the breads and cakes and inside the bread and cakes were followed by thermo sensors combined with a logger (7 m SSU type T fra Ellab A/S, Hillerød, Denmark and TC-08 Picologger, Pico Technology, Cambridge, UK).

2.4. Homogenisation

All samples of eggs, raw and cooked, were homogenised in a Waring Blender until homogenity, 5 s for raw eggs and 15–20 times 1 s for cooked eggs. Breads and cakes were homogenised in a food processor (Tecator 1094 Homogenizer, Foss Tecator, Höganäs, Sweden). The duration of mixing was 1 min for cakes, 1 min for wheat bread, and 2 min for rye bread.

As a precaution against oxidation, all homogenizer instruments were de-aerated with nitrogen before the homgenisation process was initiated. Homogenised samples were stored at maximum −20 °C until analysis within 1 week.

2.5. Design

Each combination of food and cooking were performed three times, independently of each other, e.g. boiled eggs were heated at three different times. All analytical results were found by single determination of vitD.

2.6. Analytical method and standards

2.6.1. Vitamin D

The analytical method and the equipment used to determine $vitD_3$ and $25OHD_3$ are published elsewhere (Jakobsen, Clausen, Leth, & Ovesen, 2004; Jakobsen, Maribo, Bysted, Sommer, & Hels, 2007). In short, the internal standards of $vitD_2$ and $25OHD_2$ were added to the test sample for analyses of $vitD_3$ and $25OHD_3$, while $vitD_3$ was used for internal standard in the analyses for $vitD_2$. The samples were saponified, liquid/liquid extracted, cleaned-up in a solid-phase step, followed by one or two preparative normal phase HPLC-steps. For the final separation, detection and quantification reversed phase chromatography coupled to UV- and DAD-detectors was used.

Sample sizes taken for analysis were 20 g for eggs, 5 g for margarine, 20 g for cakes, 10 g for dough, 5 g for rye bread and 20 g for wheat bread. The precision of the $vitD_3$ analysis was determined in eggs ($n = 12$), margarine ($n = 3$), cakes ($n = 3$), and flour ($n = 7$) at 6.3%, 2.8%, 6.7%, and 5.2%, respectively. The precision for $25OHD_3$ in eggs was 6% ($n = 3$) and for $vitD_2$ in bread 5% ($n = 5$). Furthermore, the accuracy was continuously checked by participation in external quality assessment schemes (FAPAS) showing z-score <1, and analysis of the certified reference material CRM421 (14.0 μg $vitD_3$/100 g in accordance with the certification value at 14.3 ± 0.8 μg $vitD_3$/100 g). In both tests, the material was milk powder.

2.6.2. True retention

Samples were weighed (at least 5 significant digits) before and after heat treatment. These data were combined with the amount of vitD in the raw i.e. unheated samples, and the amount of vitamin D in heat treated samples, respectively, to calculate true retention. In the calculation, the amount of cooked food is the amount of raw food after heat treatment (Murphy, Criner, & Gray, 1975).

$$\%\text{True retention} = \frac{(\mu\text{g vitD per 100 gram of cooked food multiplied by the amount of cooked food}) * 100}{(\mu\text{g vitD per 100 gram of raw food multiplied by the amount of raw food})} \quad (1)$$

2.7. Statistical test

Two-way ANOVA was used to test if heating procedure and vitamin D compound influenced retention in eggs ($vitD_3$ and $25OHD_3$) and in bread ($vitD_3$ and $vitD_2$). One-way ANOVA was used to test the retention of vitamin D_3 in margarine as the only ingredient and as ingredient in cake.

Least Significance Difference was used to test for differences between the heating procedures, while *t*-tests were used to test, if the retention differed from 100% (Excel 2010). *P*-value at minimum 0.05 was classified as a significant difference. All results are given as average ± sd.

3. Results

The vitD contents found in the raw samples of the different foodstuffs are shown in Table 1. The results for the retention of vitD in the cooked foodstuffs, i.e. egg, margarine and bread are presented in Tables 2–4, respectively. The tables show the conditions under which the different food was prepared, the weight loss measured from raw to cooked food product, and where appropriate the true retention of $vitD_3$, $vitD_2$, and $25OHD_3$. The temperature measured inside cakes and breads throughout the baking process are shown in Fig. 1.

Table 1
Content of vitamin D vitamers in raw samples. All treatments $n = 3$.

μg/100 g raw sample				
	$VitD_3$	$25OHD_3$	$VitD_2$	$25OHD_2$
Eggs	1.10 ± 0.03	1.21 ± 0.02	<0.05	<0.05
Margarine	13.5 ± 0.4	n.a.	n.r.	n.r.
Cakes	1.71 ± 0.12	n.a.	n.a.	n.a.
Wheat bread ($VitD_3$)	10.8 ± 0.3	n.r.	n.r	n.r.
Wheat bread ($VitD_2$)	n.r.	n.r.	9.5 ± 0.4	n.r.
Rye bread ($VitD_3$)	8.7 ± 0.3	n.r.	n.r	n.r.
Rye bread ($VitD_2$)	n.r.	n.r.	8.5 ± 0.4	n.r.

n.a.=not analysed; n.r.= not relevant.

No significant difference in retention was found between $vitD_3$ and $25OD_3$ in eggs, but the preparation condition affected the degree of retention ($p < 0.05$). There was 39% retention of $vitD_3$ and $25OHD_3$ in eggs heated in the oven for 40 min, significantly different from 100%. On average the retention in boiled and scrambled eggs were 82–88% for both vitamers. It was significantly different from 100% ($p < 0.05$) for $25OHD_3$ in scrambled eggs.

Preparation of margarine, fried in a pan or heated in an oven, showed 82% and 45% retention of $vitD_3$, significantly different from 100%. A significant difference in retention between the two cooking procedures was also found. When using margarine as an ingredient, the retention of 64% for $vitD_3$ in a cake baked for 60 min was significantly different from 100%. Also, the retention of $vitD_3$ in cakes was not significantly different from heating the margarine in a pan or in an oven.

The recommended baking time for the wheat bread was 30 min, half the wheat bread was baked for an additional 30 min, these bread turned out burned with a very hard crust indicating a serious "over-baking". In the wheat and the rye bread, a significant difference was found between the retention of $vitD_2$ and $vitD_3$. Furthermore, the retention of $vitD_3$ and $vitD_2$ in rye bread of 69% and 73%, respectively, was significantly different from the retention of 85% and 89% in wheat bread baked for 30 min. The final temperature measured (104 °C) inside the wheat bread baked for 60 min at approx. 170 °C was significantly higher than inside the rye bread (90 °C) baked for 60 min at approx. 186 °C.

4. Discussion

When calculating the retention of nutrients during cooking, it should be prioritized to use true retention factors, i.e. based on calculation of weight and content of the nutrient in the raw and the processed food, compared to apparent retention factors, i.e. content of the nutrient calculated on dry weight, wherever possible (Murphy et al., 1975). Furthermore, the tools used in the investigation of retention of vitD during preparation of the different food should be carefully evaluated in terms of the analytical methods applied, and the information of the preparation conditions e.g. temperature, time, sizes of pans and pots.

Table 2
Eggs – True retention of vitamin D_3 and 25-hydroxyvitamin D_3. All treatments $n = 3$.

	Preparation conditions		Eggs			
	Time	Temperature	Final temp	Weight loss	True retention	
	min	°C	°C	%	%VitD_3	%25OHD_3
Eggs, boiled, pot	10	100	72 ± 2	n.r.	88 ± 7^{a}	86 ± 6^{a}
Eggs, scrambled, pan	3	n.a.	68 ± 2	14.3 ± 0.9	82 ± 13^{a}	84 ± 2^{a*}
Eggs, heated, oven	40	159 ± 9	87 ± 2	32.8 ± 0.8	39 ± 8^{b*}	39 ± 3^{b*}

n.a. = not analysed; n.r.= not relevant. Different letters (a,b) in a column indicate significant differences ($p < 0.05$). A star (*) indicate a retention significant from 100% ($p < 0.05$).

Table 3
Margarine – True retention of vitamin D_3. All treatments $n = 3$.

	Preparation conditions		Margarine/cake		
	Time	Temperature	Final temp	Weight loss	True retention
	min	°C	°C	%	%VitD_3
Margarine, pan	3	n.a.	124 ± 7.9	29.5 ± 0.8	82 ± 4^{a*}
Margarine, oven	40	166 ± 9	138 ± 2.1	28.3 ± 0.1	45 ± 7^{b*}
Cakes, oven	60	172 ± 13	101 ± 1.4	6.5 ± 1.1	64 ± 14^{ab*}

n.a. = not analysed. Different letters(a,b) in a column indicate significant differences ($p < 0.05$). A star (*) indicate a retention significant from 100% ($p < 0.05$).

Table 4
Wheat bread and rye bread – True retention of vitamin D_3 and vitamin D_2. All treatments n = 3.

	Preparation conditions		Bread	VitD_3 enriched bread		VitD_2 enriched bread	
	Time	Temperature	Final temp	Weight loss	Retention	Weight loss	Retention
	min	°C	°C	%	%VitD_3	%	%VitD_2
Wheat bread, oven	30	170 ± 8	94 ± 3^{ab}	7.6 ± 0.4	85 ± 1^{a*}	6.8 ± 0.4	89 ± 3^{a*}
Wheat bread, oven$^{¤}$	60	170 ± 8	104 ± 4^{a}	13.5 ± 0.7	85 ± 4^{a*}	13.7 ± 1.0	85 ± 2^{ab*}
Rye bread, oven	60	186 ± 9	90 ± 2^{b}	7.3 ± 0.2	69 ± 2^{b*}	7.0 ± 0.0	73 ± 4^{b*}

Baked additional 30 min compared to recommended in recipe. Different letters (a,b) in a column indicate significant differences ($p < 0.05$). A star (*) indicate a retention significant from 100% ($p < 0.05$).

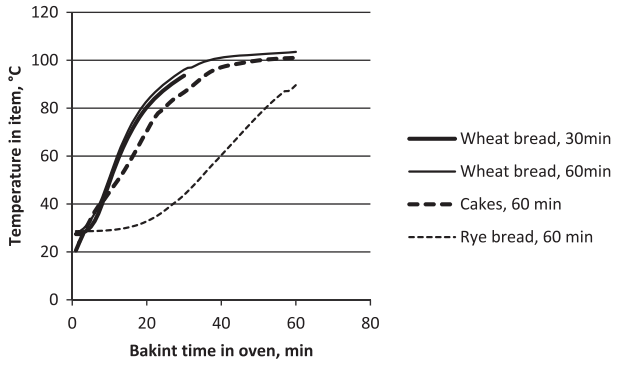

Fig. 1. The temperature measured inside cakes and breads throughout the baking process.

Essential for retention studies is the application of an analytical method with a high precision, and an appropriate limit of quantification. We aimed at investigating the true retention of vitD performing the analysis with an inter-day precision between 3% and 7% for the four foodstuffs tested. Furthermore, in our design we included appropriate control of the temperature used.

The first study on retention of vitD was in beef, it found retention levels at around 35–42%. However, the study included no information on the preparation process, and the analytical method applied did not use the mandatory internal standard for quantification and showed unsatisfactory separation to interferences (Bennink & Ono, 1982). Since then investigations of true retention of vitD in fish and pork have shown negligible loss of vitD_3 and 25OHD_3 by cooking or smoking, and of vitD_2 in fried mushrooms (Bhuiyan, Ratnayake, & Ackman, 1993; Clausen, Jakobsen, Leth, & Ovesen, 2003; Mattila, Ronkainen, Lehikoinen, & Piironen, 1999). An investigation of cooked salmon indicated a similar high retention (Elmadfa et al., 2006).

Our results for retention of vitD_3 and 25OHD_3 in boiled and scrambled eggs were between 82% and 88%. For scrambled eggs the retention for 25OHD_3 at 84% was significantly different from 100%. This significance was due to a low uncertainty (sd = 2%). Former studies found no significant loss when heating egg yolks and eggs (Mattila et al., 1999; Roe, Pinchen, Church, & Finglas, 2012). We found a significant loss of vitamin D in eggs. Eggs heated in the oven for 40 min at 160 °C had retention of 39% for vitD_3 and 25OHD_3, significantly different from 100%. Our findings are supported by earlier studies that observed instability of vitamin D_3 during heating of pure vitamin D_3 (Chen, Raymond Terepka, Lane, & Marsh, 1965).

To our knowledge, we are the first to study the retention of vitD_3 in fortified margarine in household cooking. The weight loss in margarine at 28–30% could be explained by a fat percentage of 70%. The retention in margarine when frying it for 3 min in a pan at 82% and heating it in the oven for 40 min at 45% was significantly different from 100% and significantly different from each other. The retention of vitD_3 derived from margarine, as an ingredient in cakes, was significantly different from 100%. But, the results showed no difference in retention from frying or preparation in the oven due to a high variation in the results for cakes.

Studies investigating the retention of vitD$_3$ in bread have not shown identical results (Madsen et al., 2013; Natri et al., 2006). In our study we compared traditional vitD$_3$ fortification of bread with biofortified vitD$_2$ yeast. A previous study investigated pure substances and identified higher stability of vitD$_2$ than of vitD$_3$ under humid condition at 40 °C (Grady & Thakker, 1980). We found a significant but negligible, slightly higher retention of vitD$_2$ compared to vitD$_3$ in bread baked at 170–186 °C. No significant decrease of vitD in wheat bread was found when the baking time was extended from 30 to 60 min. Irrespectively of the baking time, the retention of vitD in wheat bread was significantly different from 100%. Comparing wheat bread baked, according to the recipe, for 30 min with rye bread, we found a significantly lower level of retention in rye bread. The temperature curves during baking of wheat bread and cakes were almost alike, while the slope for the temperature in rye bread was less steep (Fig. 1). Despite a higher oven temperature for rye bread than for wheat bread, the final temperature in the rye bread was significantly lower than in the wheat bread baked for 60 min. Hence the temperature cannot explain the lower retention in rye bread. Rye bread has a lower pH than wheat bread, which might be part of the explanation for the lower retention due to the known acidic isomerisation of vitD to isotachysterol (Agarwal, 1990; Jin et al., 2004; Seamark et al., 1980).

In the design we have attempted during cooking to follow the temperature of the heating device and of the food item. However, we may emphasize that the temperature measured in the bread and cakes was obtained in the middle of the food item, which means that higher temperatures will appear in the crust or between the crust and the middle. The theoretical knowledge of the isomerisation of vitD in acidic environment and at temperatures above 100 °C combined with the sensitivity of vitD metabolites to oxidation are supposed to be the explanation of the observed low retention of vitD metabolites. When eggs and margarine were fried for 3 min in a pan and heated in an oven for 40 min, we found similar retention levels of vitD$_3$ in enriched margarine and in non-enriched eggs. For both foodstuff, retention levels when frying were at 82% and the preparation in the oven resulted in a level of approx. 40%. These results indicate that the content of vitD in eggs bound to protein or present as ester does not protect against degradation during heating.

The retention of vitD in household cooking should be taken into account in the calculation of dietary intake of vitD. Additional research is needed before recommendations can be given to the consumer on the most advantageous cooking methods regarding optimal retention of vitD.

5. Conclusion

The retention of vitamin D compounds in eggs and margarine during heat treatment in an oven for 40 min at normal cooking temperature showed retention at 39–45%, while frying resulted in retention at 82–84%. Boiled eggs were found to have a similar level of retention (86–88%). For bread baked, as recommended in the recipe, the retention of vitamin D$_3$ in rye bread at 69% was lower than the retention in wheat bread at 85%. A similar observation was made for vitamin D$_2$, although the retention was slightly higher, 73% and 89%. No difference between retention of vitamin D$_3$ and 25-hydroxyvitamin D$_3$ in eggs was shown.

Cooking may cause detrimental loss of vitamin D, but it depends on the actual foodstuffs and the heating process. Further research is needed to optimise cooking procedures to enhance retention of vitamin D. Vitamin D retention should be taken into account in future calculations of dietary intake of vitamin D.

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