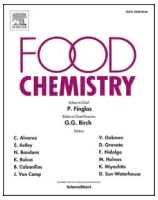
Food Chemistry 397 (2022) 133839

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



# Impact of cooking on vitamin $D_3$ and 25(OH)$D_3$ content of pork products

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ARTICLE INFO

*Keywords:*
Cholecalciferol
Loin
Mince
Sausage
Retention
Heat

ABSTRACT

Little is known regarding the impact of cooking on vitamin D content in pork, despite meat being a major contributor to vitamin D intakes.

This paper investigated the effect of household cooking (pan-fry/roast/grill/sous-vide/sauté), on the vitamin $D_3$ and 25-hydroxyvitamin $D_3$ (25(OH)$D_3$) concentration/retention in pork loin, mince and sausages. We hypothesised that vitamin D concentrations would be higher in cooked vs raw pork, and retention would differ between products.

Cooking significantly increased vitamin $D_3$ (+49 %) and 25(OH)$D_3$ (+33 %) concentrations. All cooked loin vitamin $D_3$ concentrations were significantly lower than mince/sausage. Vitamin $D_3$ retention was > 100 % for all samples (102–135 %), except sauté mince (99 %) which still did not differ significantly from 100 % retention. Sous-vide cooking resulted in the highest vitamin $D_3$ retention (135 %).

Likely owing to water/fat loss, household cooking of pork results in favourable retention of vitamin $D_3$ and 25(OH)$D_3$. The type of pork product has greater influence than cooking method.

## 1. Introduction

Vitamin D (calciferol), a prohormone and fat-soluble sterol, exists in two main isoforms: vitamin $D_3$ (cholecalciferol) and vitamin $D_2$ (ergocalciferol). Vitamin $D_3$ is mainly present in foods of animal origin such as eggs, oily fish, red meat, liver and dairy whilst vitamin $D_2$ can be obtained from plant-based foods including mushrooms, bread baked with ultraviolet (UV)-treated yeast and some fortified products. When consumed, biologically inert vitamin D is sequentially hydroxylated by the liver and kidneys to 25-hydroxyvitamin D (25(OH)D) and the final active metabolite 1,25-dihydroxyvitamin D (1,25$(OH)_2$D), respectively (Holick, MacLaughlin, & Doppelt, 1981). In humans, the majority of vitamin D is derived from the action of UVB radiation (wavelength 290–315 nm), rather than food (Holick, 2007). However, a plethora of environmental and personal factors may diminish the action of UVB light and capacity of the skin to produce vitamin $D_3$ (Fink, Peters, Koplin, Brown, & Allen, 2019; Nair & Maseeh, 2012; Tsiaras & Weinstock, 2011) Thus, many populations remain reliant on dietary sources. Evidence consistently shows that vitamin D status in the UK is suboptimal, owing to low dietary intakes and limitations in endogenous synthesis from sunlight (England, 2020a). Therefore, deficiency of vitamin D is prevalent and of global public health concern (Cashman et al., 2016). Severe and prolonged vitamin D deficiency may manifest as osteomalacia in adults and rickets in children (Holick, 2005; Institute of Medicine, 2011).

Meat and meat products are the main contributor to vitamin D intakes in UK adults (25 %) and teenagers (29 %), providing both vitamin $D_3$ and 25(OH)$D_3$ (England, 2020b), albeit the latter is less well quantified in foods. In addition, vitamin D is susceptible to degradation following heat, light, oxygen and moisture exposure (Maurya, Bashir, & Aggarwal, 2020). The presence of fat affects the stability of vitamin D in food and therefore synthetic and natural emulsifying agents, such as milk proteins, have been added to increase vitamin D stability (Guttoff, Saberi, & McClements, 2015; Kazmi, Vieth, & Rousseau, 2007; Tipchuwong, Chatraporn, Ngamchuachit, & Tansawat, 2017; Tippetts, Martini, Brothersen, & McMahon, 2012; Wagner, Sidhom, Whiting, Rousseau, & Vieth, 2008; Ziani, Fang, & McClements, 2012). These influences from processing and storage, coupled with natural variations in food composition, means nutritional values on labelled meat containing produce may not always be precise. Furthermore, when considering

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https://doi.org/10.1016/j.foodchem.2022.133839
Received 18 March 2022; Received in revised form 25 July 2022; Accepted 29 July 2022
Available online 2 August 2022

human consumption, the amount of vitamin D within cooked meat may not necessarily reflect its bioavailability due to it being bound within the food matrices, and depending on the quantity and complexity of the consumed food (Maurya & Aggarwal, 2017). This is important for industry to avoid inadvertently misleading consumers. Notably, the use of a health claim marketed on a food item is based upon the concentration of parental vitamin D in food as consumed (EFSA, 2006); hence, the impact of various common household cooking methods is of clear significance. Transparency regarding the cooking methods employed, if any, may better inform consumers regarding the vitamin D concentration displayed in the nutritional information panel. Opportunity may also exist for industry to advise consumers on the optimal cooking method to retain vitamin D. This is particularly relevant for biofortified meat, whereby vitamin D is naturally increased by UVB exposure and/or feed supplementation (Neill, Gill, McDonald, McRoberts, & Pourshahidi, 2021). If research and resources are dedicated to biofortification on-farm implementation, it would be amiss to potentially lose this increased vitamin D concentration post-cooking.

Little is known regarding the impact of common household cooking on vitamin D retention in meat. Previous research has investigated vitamin D retention in eggs, margarine, bread, fish and mushrooms (Jakobsen & Knuthsen, 2014; Ložnjak & Jakobsen, 2018) with heterogenous results. Evidently, the type of foodstuff and cooking method impact vitamin D retention and stability, potentially causing detrimental losses. Owing to limited vitamin D sources and numerous factors influencing endogenous production, resulting in prevalent rates of deficiency, it is therefore important to minimise vitamin D losses during cooking of meat.

The aim of the current work was to investigate the effect of different cooking methods, usually performed in households, on the vitamin D concentration and retention in pork loin chop, mince and sausages. It was hypothesised that vitamin D concentrations would be higher in processed pork vs loin and following cooking.

## 2. Material and methods

### 2.1. Pork samples

To replicate household cooking methods, pork products were purchased from three local butchers in Northern Ireland (Lisburn; Belfast; Portstewart). An overview of the experimental study design is shown in Fig. 1. Loin, mince and sausage were selected as raw pork products owing to popularity amongst consumers. Two boneless pork loin chops were each cut into 14 slices approximately 2 cm in thickness (right to left from the head), and the first and last loin slices were discarded. The remaining 12 slices were allocated sequentially to cooking treatments. For example, slices 1, 5 and 9 of each loin were assigned to pan-fry and slices 2, 6 and 10 were assigned to roast cooking. One-third of each loin slice was cut to be kept raw ($n = 24$), and the remaining two-third of the loin slice was cooked ($n = 24$). Pork mince was separated in to approximately 200 g samples for sautéing ($n = 6$ portions), with a raw subsample set aside ($n = 6$ portions). Pork sausages were cooked as purchased ($n = 6$ pan-fry; $n = 6$ grill) with additional sausages kept for raw analysis ($n = 6$). Each cooking treatment was performed in duplicate e.g. three samples of roasted loin were cooked on two different occasions.

### 2.2. Cooking treatments

The seven different cooking/product treatment groups were as follows: pan-fry, roast, grill, sous-vide loin, sauté mince, pan-fry and grill sausage (Table 1). These cooking methods were selected to reflect the most common household cooking scenarios for each pork product to therefore provide real-life relevance. Three pork samples were cooked in each treatment and performed in duplicate from November 18, 2019 to November 22, 2019. Loin samples were cooked with fat on and then removed, as per common consumer practice. The entirety of the experimental design was conducted at Agri-Food and Biosciences Institute (AFBI, Belfast, Northern Ireland). No additional ingredients were added to any treatments, with the exception of pan-frying where 5 g vegetable oil (Tesco, Hertfordshire, UK) was added to the pan before

**Table 1**
Summary specification of pork cooking methods ($n = 6 \times 7$ treatments).

| Pork product | Cooking method | Temperature | Time, approx. min* |
|---|---|---|---|
| Loin | Pan-fry (+5 g vegetable oil) | Medium | 10 |
| | Roast (uncovered) | 180 °C | 15 |
| | Grill | Medium | 15 |
| | Sous-vide† | 60 °C | 60 |
| Mince | Sauté | Medium | 4–6 |
| Sausage | Pan-fry (+5 g vegetable oil) | Medium | 10 |
| | Grill | Medium | 12 |

*Samples were turned halfway through cooking when appropriate. †After sous-vide treatment, loin was pan-seared for 1 min both sides to replicate common household cooking scenarios. *n*, number of samples; g, grams; min, minutes.

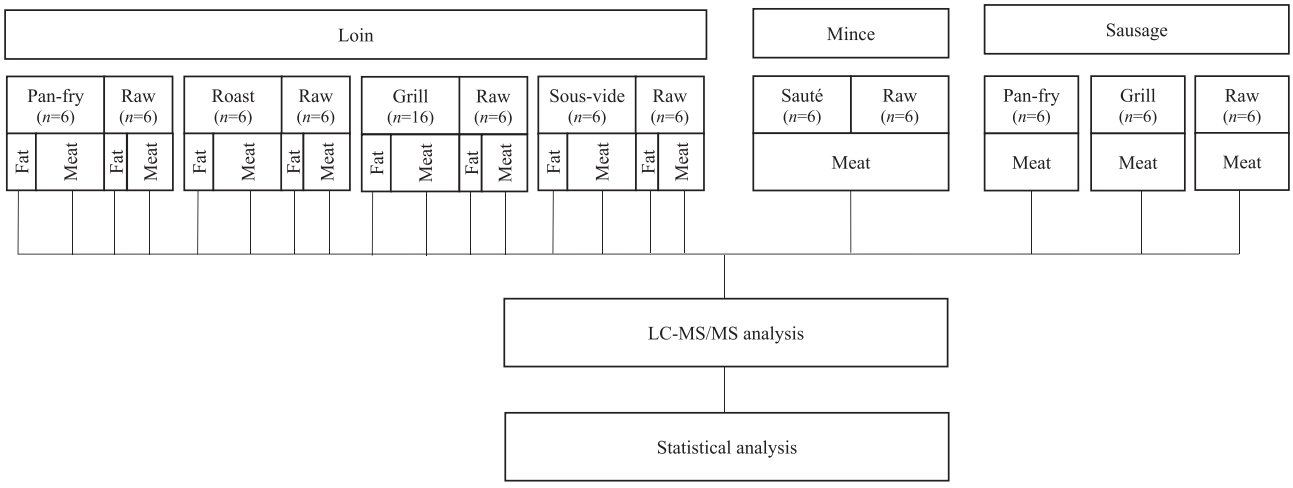


**Fig. 1.** Flow diagram of experimental study design. Cooking was conducted in duplicate ($n = 3$ samples $\times$ 2 occasions, therefore total $n = 6$ shown in figure). Loin slices ($n = 2$ loin; $n = 24$ slices) were allocated sequentially to cooking treatments. For example, slices 1, 5 and 9 of each loin were assigned to pan-fry and slices 2, 6 and 10 were assigned to roast cooking. Pork products were purchased from three local butchers in Northern Ireland (Lisburn; Belfast; Portstewart). *n*, number of samples; LC-MS/MS, liquid chromatography-tandem mass spectrometry.

cooking to replicate household cooking scenarios. All sausage samples were pricked prior to cooking. Roasted loin was oven-baked (180 °C; 356 °F; Rational AG, iCombi Classic Combi Oven, Landsberg am Lech, Germany) in uncovered individual foil trays. An electric skillet (Prestige, Meyer Group Ltd., Wirral, United Kingdom) was used to pan-fry loin and sausage, and sauté mince. A non-contact infrared thermometer (STTMProPlus, Raytek, Thermimport Quality Control, Zevehuizen, Netherlands) measured the consistent temperature of the skillet pan before and during cooking. Samples of loin and sausage were grilled using a conventional oven (Zanussi, Electrolux, XCE7702X, 230–240 V, 50 Hz, 10.5–11.4 kW, Britain). For sous-vide treatment, loin samples were vacuum sealed (La.va, Vakuumverpackung, V.500 Premium Lime VL500P, Manfred landig, Valentintraβe, Saulgau) and set in a thermostatic water bath (Buffalo Bain Marie SP02680, Buffalo Appliances, UK). Afterwards, sous-vide samples were pan-seared for 1 min either side to imitate household cooking. All meat samples were cooked to an internal temperature of 65 °C (149 °F), measured using a digital thermometer (Portable K-Thermocouple, Hanna Instruments, Bedfordshire, UK).

### 2.3. Weighing and homogenisation

Raw and cooked pork loin chop samples were weighed (Sartorius, Göttingen, Germany) with and without fat (to at least 2 decimal places). Mince and sausages were weighed pre and post-cooking. Their respective raw samples were also weighed. As fat is incorporated within mince and sausage products, only loin meat samples had fat trimmed and analysed. Raw and cooked samples were homogenised when cool using a mini hand blender chopper (Cookworks, Argos Ltd., United Kingdom) for approximately 20 s and then dispensed in to sealed plastic bags. Samples were stored at −20 °C until analysis.

### 2.4. Vitamin D analysis

Parent vitamin D (vitamin $D_3$ and $D_2$) and hydroxy metabolites (25$(OH)D_3$ and 25$(OH)D_2$) were quantified in raw and cooked pork meat (5 g), and raw and cooked pork fat (3 g) using liquid chromatography with tandem mass spectrometry (LC-MS/MS). Analysis of meat ($n = 78$) and fat ($n = 48$) was conducted in duplicate extractions, except when the quantity of fat was insufficient, in which case it was analysed singly. Where possible, cooking juices were collected during or after heat treatments ($n = 30$), however, quantities were limited which led to poor replication between duplicates and concentrations below the level of detection (LOD). Therefore, these results have been largely omitted and the focus of analysis was quantifying vitamin D from foods consumed due to its relevance for consumers and commercialisation. The analytical method was adapted from previously published methods (Ding et al., 2010; Strobel, Buddhadasa, Adorno, Stockham, & Greenfield, 2013; Trenerry, Plozza, Caridi, & Murphy, 2011). A full description of the method is provided in Supplemental Material. In brief, homogenised samples were added to 15 ml ethanol, 15 ml distilled water, 6 g potassium hydroxide (KOH) and 0.5 g antioxidant (ascorbic acid) before flushed with nitrogen and placed in an orbital incubator (Gallenkamp, Cambridge, UK) shaking at 125 rev/min and held at 25 ± 2 °C for at least 16 h. The saponified extract was transferred to a chem-elut cartridge (50 ml, Agilent Technologies UK Ltd, Cheshire, UK) and allowed to absorb (~15 min) before eluted with petroleum ether (50 ml × 3), concentrated to dryness using a Thermo React-therm under a gentle nitrogen stream (Thermo Scientific, Massachusetts, United States) and then reconstituted in 1 ml hexane. Extracts were then centrifuged at 3000 rpm for 10 min (Sigma 3–15 k Centrifuge). Samples were cleaned-up by solid-phase extraction (SPE) using hexane and hexane: ethyl acetate (90:10 v/v). Vitamin $D_2$ and vitamin $D_3$ were eluted with hexane: ethyl acetate (80:20). 25$(OH)D_2$ and 25$(OH)D_3$ were eluted and collected as a separate fraction with hexane: ethyl acetate (60:40). Each fraction was evaporated to dryness and reconstituted in 500 μl methanol. Extracts were derivatised with 4-phenyl-1,2,4-triazole-3,5-dione (PTAD) (5 mg/ml) in acetonitrile and allowed to react for at least an hour before LC-MS/MS analysis. The derivatized extracts (10 μl injection) were separated on a Nexera X2 (Shimadzu UK Limited, Milton Keynes, UK) UHPLC/HPLC system (Sciex, Cheshire, UK). For MS/MS analysis a 6500 QTRAP triple quadrupole ion trap mass spectrometer with an Iondrive Turbo V source (Sciex, Cheshire, UK) was used and the instrument operated in electrospray ionisation (ESI) positive ion multiple reaction monitoring mode (MRM). Data acquisition was carried out using Analyst software (AB Sciex) version 1.6.2 with data processing and quantitation carried out using MultiQuant software (AB Sciex) version 3.0. Calibrations curves were constructed by plotting the peak response ratio for each analyte against the corresponding stable labelled internal standard verses the corresponding concentration and fitting the data using linear regression with a weighting factor of 1/x. The limit of quantification (LOQ) and LOD for meat was 0.02 μg/kg and 0.01 μg/kg. For fat, the LOQ and LOD was set at 0.033 μg/kg and 0.017 μg/kg, respectively. Standard curve correlation coefficients were greater than 0.99 for all analytes. Analytical recovery was determined on the basis of the expected value as calculated from the spiked recovery samples. The mean recoveries ranged from 86.55 to 97.65 % and 85.37–100.58 % for vitamin $D_3$ and 25$(OH)D_3$, respectively. All analyses were conducted at AFBI headquarters, Belfast.

### 2.5. Weight loss and true retention

Percentage weight loss of meat was calculated. Weight data was combined with vitamin D concentrations (μg/kg) in raw and cooked meat to calculate percentage true retention (see Eq. (1)) (Murphy, Criner, & Gray, 1975).

$$\text{True retention (\%)} = (\text{vitamin D content of cooked meat (μg/kg)} \times \text{weight of cooked meat (kg)})/(\text{vitamin D content of raw meat (μg/kg)} \times \text{weight of raw meat (kg)}) \times 100 \quad (1)$$

### 2.6. Statistical analysis

All statistical analyses were conducted using the Statistical Package for the Social Sciences (IBM SPSS Statistics 25, Chicago, IL, USA). First, normality of data was checked using Shapiro-Wilk tests and subsequently one-way ANOVA was used to test if cooking method influenced vitamin D concentrations in raw and cooked pork meat and fat. Paired *t*-test assessed differences between raw and cooked products, and compared true retention to 100 %. Correlations between true retention (vitamin $D_3$ and 25$(OH)D_3$) and weight loss were performed by using Spearman's correlation coefficients. Total vitamin D activity (μg/kg) was calculated as shown in Eq. (2). For all analyses, $p < 0.05$ was considered statistically significant.

$$\text{Total vitamin D activity}(\mu g/kg) = \text{vitamin } D_3 + (25(OH)D_3 \times 5) \quad (2)$$

## 3. Results

The vitamin $D_3$, 25$(OH)D_3$ and total vitamin D activity concentrations in raw and cooked samples of pork meat are presented in Table 2. Vitamin $D_3$, 25$(OH)D_3$ and total vitamin D activity concentrations significantly increased from raw and cooked meat samples for all cooking treatments ($p < 0.05$). No significant differences in vitamin D concentrations were observed between the different cooking methods for pork loin chop meat samples, but these were significantly lower than that reported for cooked mince and sausages. The cooking method did not have any effect on vitamin $D_3$, 25$(OH)D_3$ or total vitamin D activity concentrations between the two sausage groups (pan-fry and grill). Vitamin $D_3$ concentrations in sautéed mince differed from all cooking methods and samples (higher than loin but lower than sausages) but differed only from loin samples when considering 25$(OH)D_3$

**Table 2**
Vitamin D concentration (μg/kg) in pork meat pre and post different home cooking methods ($n = 6$ per treatment).

| Pork product | Cooking method | Vitamin $D_3$ (μg/kg) | | 25(OH)$D_3$ (μg/kg) | | Total vitamin D activity (μg/kg) | |
|---|---|---|---|---|---|---|---|
| | | Raw meat | Cooked meat* | Raw meat | Cooked meat* | Raw meat | Cooked meat* |
| Loin | Pan-fry | $1.11 \pm 0.40^a$ | $1.66 \pm 0.40^a$ | $0.88 \pm 0.14^a$ | $1.19 \pm 0.11^a$ | $5.51 \pm 1.03^a$ | $7.61 \pm 0.88^a$ |
| | Roast | $0.98 \pm 0.22^a$ | $1.39 \pm 0.23^a$ | $0.85 \pm 0.08^a$ | $1.06 \pm 0.14^a$ | $5.25 \pm 0.56^a$ | $6.69 \pm 0.93^a$ |
| | Grill | $0.84 \pm 0.11^a$ | $1.44 \pm 0.10^a$ | $0.79 \pm 0.04^a$ | $1.10 \pm 0.08^a$ | $4.78 \pm 0.32^a$ | $6.94 \pm 0.45^a$ |
| | Sous-vide | $0.92 \pm 0.12^a$ | $1.67 \pm 0.33^a$ | $0.80 \pm 0.05^a$ | $1.10 \pm 0.11^a$ | $4.92 \pm 0.35^a$ | $7.15 \pm 0.75^a$ |
| Mince | Sauté | $2.59 \pm 0.52^b$ | $3.39 \pm 0.32^b$ | $1.14 \pm 0.22^b$ | $1.46 \pm 0.15^b$ | $8.31 \pm 1.56^b$ | $10.70 \pm 1.02^b$ |
| Sausage | Pan-fry | $4.14 \pm 0.20^c$ | $5.58 \pm 0.93^c$ | $1.09 \pm 0.09^b$ | $1.41 \pm 0.09^b$ | $9.59 \pm 0.34^b$ | $12.66 \pm 1.31^c$ |
| | Grill | $4.14 \pm 0.20^c$ | $5.49 \pm 0.88^c$ | $1.09 \pm 0.09^b$ | $1.46 \pm 0.07^b$ | $9.59 \pm 0.34^b$ | $12.79 \pm 1.16^c$ |

Data presented as mean ± SD of $n = 6$ independent experiments ($n = 3$ samples × 2 occasions) analysed in duplicate. Values not sharing a common superscript letter in columns (a, b, c) are significantly different ($p < 0.05$) between cooking methods; one-way ANOVA and Tukey post hoc test. *Within rows, all cooked meat values were significantly higher than raw meat ($p < 0.01$); paired *t*-test. Significance set at $p < 0.05$ throughout. *n*, number of samples; 25(OH)$D_3$, 25-hydroxyvitamin $D_3$; SD, standard deviation. Total vitamin D activity (μg/kg) = vitamin $D_3$ + (25(OH)$D_3$ × 5).

concentrations. Total vitamin D activity in mince and sausage samples only differed significantly after cooking.

Table 3 shows the vitamin $D_3$, 25(OH)$D_3$ and total vitamin D activity concentrations in raw and cooked pork loin chop fat. There was no significant difference between cooking methods, or between raw and cooked values, with the exception of sous-vide with higher vitamin $D_3$ and total vitamin D activity in cooked vs raw fat.

The percentage weight loss from raw to cooked pork and the true retention of vitamin $D_3$, 25(OH)$D_3$ and total vitamin D activity are shown in Table 4. Vitamin $D_3$ retention was significantly greater than 25(OH)$D_3$ only in grill loin and sous-vide loin ($p < 0.05$). The cooking method and type of pork product affected the degree of retention. On average, the range of vitamin $D_3$, 25(OH)$D_3$ and total vitamin D activity retention was 99–135 %, 88–129 % and 90.5–130.3 %, respectively. Sous-vide loin samples had the greatest vitamin $D_3$ retention (135 %) which was significantly higher than that for roasted loin (102 %) and sautéed mince (99 %). Pan-fried sausages had the greatest 25(OH)$D_3$ retention (129 %) and was significantly different to roasted loin (88 %), grilled loin (106 %), sous-vide loin (102 %) and sautéed mince (95 %). The retention in sausages was not significantly different between pan-fry and grill cooking. For total vitamin D activity retention, roast loin was significantly lower than both sausages, while sauté mince was significantly lower than pan-fried sausage. A significant correlation was observed between vitamin $D_3$ and 25(OH)$D_3$ true retention ($r = 0.672$, $p < 0.001$). Weight loss following cooking amongst all samples ranged from 1 to 29 %. Considering only loin samples, the weight loss range was 21–29 %, with roast loin showing the greatest difference in weight and pan-fry cooking resulting in the least. An inverse correlation was identified between weight loss and vitamin $D_3$ and 25(OH)$D_3$ true retention ($r = -0.361$, $p = 0.019$; $r = -0.608$, $p < 0.001$, respectively).

## 4. Discussion

To our knowledge, this is the first study to investigate how vitamin D concentration in various pork products (loin, mince, sausage) are impacted by differing household cooking methods (pan-fry, roast, grill, sous-vide, sauté). Cooking significantly increased vitamin $D_3$, 25(OH)$D_3$ and total vitamin D activity concentrations in all pork products. The type of pork product appeared to have more of an effect than the cooking treatment, arguably owing to the differing fat contents with much higher concentrations present in mince and sausage compared to lean loin. Absolute vitamin $D_3$ and 25(OH)$D_3$ values showed no differences between pork loin chop cooked by different methods ($p > 0.05$); whereas vitamin $D_3$ significantly differed between cooked loin, mince and sausage, irrespective of cooking process ($p < 0.05$). Moreover, true vitamin $D_3$ retention only differed between sous-vide and roast loin samples. Raw pork meat values were generally aligned with previously published values reported in literature and national food composition databases, albeit on the lower range, owing to different cuts and analytical methods (Schmid & Walther, 2013). In good agreement with the current findings, Clausen and colleagues (2003) reported an increase in vitamin D concentrations in lean pork meat following oven roasting. However, vitamin $D_3$ concentration in raw and cooked pork in the current study (0.98 and 1.39 μg/kg) were more than double that reported in the study by Clausen, Jakobsen, Leth, and Ovesen (2003) (0.4 and 0.6 μg/kg). Roasted loin 25(OH)$D_3$ concentrations were only modestly higher in our work (0.85 vs 1.06 μg/kg). Differences may be owed to alternative cooking temperature or the use of high-performance liquid chromatography (HPLC) analysis. In recent times, and in the present study, LC-MS/MS has been the chosen analytical methodology owing to its greater specificity and sensitivity in vitamin D analyses (Shah, James, Barker, Petroczi, & Naughton, 2011).

Vitamin D retention was found to be high, and significantly higher than 100 % in a few product/cooking method combinations. Notably, meat was cooked with fat on to replicate common household cooking practices but then removed afterwards, again to replicate typical consumer eating behaviours. Potentially, vitamin $D_3$ stored within the fat, which was found to be up to 10 times higher than in the lean meat, migrated to the meat during the cooking process and thus may explain, at least in part, why retention was so high, despite weight loss being accounted for. Similarly, the 25(OH)$D_3$ concentration in fat was approximately double the levels found in lean loin meat. In addition, the

**Table 3**
Vitamin D concentration (μg/kg) in pork loin chop fat pre and post different home cooking methods ($n = 6$ per treatment).

| Pork product | Cooking method | Vitamin $D_3$ (μg/kg) | | | 25(OH)$D_3$ (μg/kg) | | | Total vitamin D activity (μg/kg) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Raw fat | Cooked fat | *p*-value* | Raw fat | Cooked fat | *p*-value* | Raw fat | Cooked fat | *p*-value* |
| Loin | Pan-fry | $9.46 \pm 5.02$ | $9.12 \pm 2.98$ | NS | $1.99 \pm 0.33$ | $1.65 \pm 0.17$ | NS | $19.41 \pm 6.40$ | $17.38 \pm 3.80$ | NS |
| | Roast | $7.46 \pm 3.54$ | $9.14 \pm 2.47$ | NS | $1.76 \pm 0.18$ | $1.63 \pm 0.18$ | NS | $16.27 \pm 4.27$ | $17.30 \pm 3.01$ | NS |
| | Grill | $6.62 \pm 3.32$ | $10.07 \pm 4.37$ | NS | $1.72 \pm 0.25$ | $1.77 \pm 0.14$ | NS | $15.22 \pm 4.10$ | $18.93 \pm 4.92$ | NS |
| | Sous-vide | $5.96 \pm 2.80$ | $10.35 \pm 4.13$ | 0.002 | $1.67 \pm 0.39$ | $1.84 \pm 0.25$ | NS | $14.30 \pm 3.56$ | $19.56 \pm 5.27$ | 0.017 |

Data presented as mean ± SD of $n = 6$ independent experiments ($n = 3$ samples × 2 occasions) analysed in duplicate. **P*-value difference within rows (raw vs cooked); paired *t*-test. Significance set at $p < 0.05$ throughout. No significant difference within columns between cooking methods for raw or cooked fat; one-way ANOVA and Tukey post hoc test (all $p > 0.05$). As fat could not be cut off from mince and sausage samples, such values are not provided. *n*, number of samples; NS, non-significant; 25(OH)$D_3$, 25-hydroxyvitamin $D_3$; SD, standard deviation. Total vitamin D activity (μg/kg) = vitamin $D_3$ + (25(OH)$D_3$ × 5).

**Table 4**
Weight loss (%) and true vitamin D retention (%) of pork products after home cooking methods ($n = 6$ per treatment).

| Pork product | Cooking method | Raw weight (g) | Cooked weight (g) | Weight loss (%) | True retention (%) | | |
|---|---|---|---|---|---|---|---|
| | | | | | Vitamin $D_3$ | 25(OH)$D_3$ | Total vitamin D activity |
| Loin | Pan-fry | 130.4 ± 7.4 | 102.7 ± 7.4 | 21.3 ± 3.1$^{a}$ | 122.5 ± 24.0$^{a,b,c}$ | 107.5 ± 9.8$^{a,b,c}$ | 110.2 ± 12.3$^{a,b,c}$ |
| | Roast | 128.3 ± 8.3 | 91.2 ± 7.2 | 29.0 ± 1.9$^{b}$ | 102.3 ± 12.9$^{a,c}$ | 88.1 ± 8.8$^{a}$* | 90.5 ± 7.7$^{a}$ |
| | Grill | 124.8 ± 16.7 | 94.3 ± 14.8 | 24.6 ± 2.8$^{a,b}$ | 129.2 ± 12.5$^{a,b,c}$* | 105.8 ± 9.5$^{a,c}$ | 109.8 ± 9.7$^{a,b,c}$ |
| | Sous-vide | 128.5 ± 18.5 | 95.6 ± 15.6 | 25.8 ± 2.2$^{a,b}$ | 134.9 ± 19.9$^{b}$* | 101.8 ± 10.3$^{a,c}$ | 108.1 ± 13.4$^{a,b,c}$ |
| Mince | Sauté | 200.1 ± 0.1 | 147.3 ± 3.2 | 26.4 ± 1.6$^{a,b}$ | 98.8 ± 16.2$^{a}$ | 95.4 ± 10.1$^{a}$ | 96.2 ± 9.8$^{a,c}$ |
| Sausage | Pan-fry | 104.4 ± 13.7 | 102.6 ± 9.4 | 1.2 ± 4.5$^{c}$ | 132.2 ± 14.5$^{b,c}$* | 128.8 ± 12.2$^{b}$* | 130.3 ± 13.1$^{b}$ |
| | Grill | 121.5 ± 9.2 | 105.8 ± 10.7 | 13.0 ± 5.2$^{d}$ | 115.4 ± 20.3$^{a,b,c}$ | 117.9 ± 19.2$^{b,c}$ | 116.8 ± 19.6$^{b,c}$ |

Data presented as mean ± SD of $n = 6$ independent experiments ($n = 3$ samples × 2 occasions) analysed in duplicate. Values not sharing a common superscript letter in columns (a, b, c, d) are significantly different ($p < 0.05$) between cooking methods; one-way ANOVA and Tukey post hoc test. *Denotes a retention significantly different from 100 % ($p < 0.05$); paired *t*-test. Loin was weighed and analysed as lean meat. No significant difference between vitamin $D_3$ and 25(OH)$D_3$ true retention in each cooking methods, except grill loin ($p < 0.001$) and sous-vide loin ($p = 0.002$); paired *t*-test. *n*, number of samples; 25(OH)$D_3$, 25-hydroxyvitamin $D_3$; SD, standard deviation. Weight loss (%) = (raw weight − cooked weight)/ raw weight × 100. Total vitamin D activity (μg/kg) = vitamin $D_3$ + (25(OH)$D_3$ × 5). True retention (%) = [vitamin D content of cooked meat (μg/kg) × weight of cooked meat (kg)]/[vitamin D content of raw meat (μg/kg) × weight of raw meat (kg)] × 100 (Murphy et al., 1975).

loin samples contained two muscles with the lower section containing greater visible fat and proving more challenging to completely remove. Therefore, some intramuscular fat of varying quantities is likely to be present in analysed lean meat. Of note, the analysed raw sample was removed from the top of the loin slice which did not contain this fattier area. Specifically considering pan-frying, vegetable oil was added to mimic typical household cooking practices and therefore oil (which may have absorbed vitamin $D_3$ from the adipose tissue) may have been absorbed during cooking and thus, impact vitamin D concentration and weight post-cooking. Previous research in pork suggests an increase in dry matter from water loss is the major determinant of increased vitamin D following cooking, despite loss of fat containing vitamin D (Clausen et al., 2003). Dry matter content was not considered in this study but should be analysed in future work to confirm this theory.

Adipose tissue is comprised of water, connective tissue and fat so natural variability exists and may offer a partial explanation for the disparity in raw fat values. Furthermore, the quantity of individual raw fat samples was generally small (range 3.39–15.61 g; mean 7.04 ± 3.20 g) meaning homogenisation was often not possible and the sample was manually cut instead which may have contributed to the observed inter and intra-variability.

Broadly in agreement with our results, Jakobsen and Knuthsen (2014) reported no difference in retention between the vitamin D metabolites (vitamin $D_3$, vitamin $D_2$, and 25-hydroxyvitamin $D_3$) after cooking eggs, margarine and bread. However, vitamin D retention in eggs (39–88 %), margarine (45–82 %) and bread (73–89 %) significantly differed from 100 % following various heat treatments (Jakobsen & Knuthsen, 2014). Jakobsen and Knuthsen (2014) concluded this may be owed to isomerisation in acidic environments and temperatures above 100 °C, in addition to oxidation sensitivity. Alternatively, in another study, the addition of lemon juice to create acidic boiling conditions seemed to preserve vitamin D in rainbow trout (water pH 4.0) and mushrooms (water pH 3.5), resulting in 20 % higher retention compared to its neutral boiling counterpart (Ložnjak & Jakobsen, 2018). Mushrooms, a natural source of vitamin $D_2$ and popular vehicle for vitamin D biofortification by UV exposure, showed mixed levels of vitamin D retention after being boiled, pan-fried or baked. (62–88 %) (Ložnjak & Jakobsen, 2018). Conversely, an older study investigating lean beef observed decreases from 1.03 μg/kg vitamin D to 0.82–0.92 μg/kg after boiling, roasting and braising, and 35–42 % retention (Bennink & Ono, 1982). However, details on the preparation process and use of internal standard during analysis are omitted. In general, research has shown negligible losses of vitamin D and overall high levels of retention in eggs, mushrooms, sunflower oil, trout, mackerel and salmon (Bhuiyan, Ratnayake, & Ackman, 1993; Elmadfa et al., 2006; Ložnjak & Jakobsen, 2018; Mattila, Ronkainen, Lehikoinen, & Piironen, 1999). Of note, not all investigated 25(OH)$D_3$ concentrations and older studies used HPLC analytical methods. Specifically, heterogeneity in the selected food samples make direct vitamin D retention comparisons challenging and data regarding cooking of pork meat is scarce.

Total vitamin D activity reported in this study are similar to those presented in the UK McCance and Widdowson's Composition of Foods Integrated Dataset (CoFID) which uses a factor of five for 25(OH)$D_3$ potency (England, 2021). Raw lean pork loin is reported as 5 μg/kg which falls within our range (4.78–5.51 μg/kg). CoFID vitamin D raw pork mince and raw pork sausages are 8 and 9 μg/kg which are in accordance with our results (8.31 and 9.59 μg/kg, respectively). No sous-vide cooking for pork loin chop or sauté mince is included within CoFID; however, it is encouraging that some different cooking methods have been considered and, in general, values are in broad agreement with the current findings. For example, we reported 6.69, 6.94, 12.66 and 12.79 μg/kg total vitamin D activity for roasted loin, grilled loin, fried sausage and grilled sausage, respectively. This is comparative to 8, 8, 11 and 11 μg/kg in CoFID. Similarly, in US and Canadian nutrient databases, raw and broiled loin was reported as ~ 3–4 μg/kg and ~ 5–6 μg/kg (U.S. Department of Agriculture, 2019; Health Canada, 2021), quantified by HPLC or LC-MS (Bilodeau et al., 2011). Notably, unlike CoFID, the 25(OH)D metabolite is not reflected in these values (Health Canada, 2015). Naturally, differences in exact cooking method, storage conditions, temperature and duration of heating process will have affected the stability of vitamin D and may, at least in part, explain modest discrepancies. Other dietary sources, beyond meat, require further investigation, particularly as fortified and, more recently, biofortified products are available. For example, it has been proposed that UV-exposed biofortified mushrooms should not be regarded as having 100 % vitamin D retention. Instead, when calculating vitamin D dietary intake from biofortified mushrooms, 75 % retention has been recommended and therefore a lower concentration contribution should be considered (Ložnjak & Jakobsen, 2018). This knowledge will ensure that intakes of vitamin D fortified and biofortified foods are not overestimated which may theoretically cause vitamin D consumption patterns to unknowingly remain suboptimal owing to inaccurate dietary calculations.

Whilst modest, grilled loin showed significantly greater vitamin $D_3$ retention compared to 25(OH)$D_3$, as did sous-vide cooking. If ratio of retention between the two metabolites (vitamin $D_3$ and 25(OH)$D_3$) differed substantially following cooking, consideration would be warranted as to the most appropriate metabolite to prioritise. Currently, only vitamin D (cholecalciferol or ergocalciferol) are considered by the European Food Safety Authority (EFSA) when marketing a vitamin D content claim (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2010). Specifically, to be labelled as a source of vitamin D, at least 15 % of the recommended nutrient intake (RNI) should be provided by the food item as consumed, meaning the cooked value is of interest rather

than the raw value (EFSA Panel on Dietetic Products, Nutrition and Allergies, 2010). Naturally this has commercial relevance, however debate exists regarding whether 25(OH)D has greater potency than vitamin D and thus, may be more effective in elevating consumer vitamin D status. Future EFSA guidance should be expanded to include 25(OH)D within total vitamin D content.

The strength of this study lies in being the first, to our knowledge, to compare vitamin D retention in a range of pork products during various cooking methods usually performed in households. Additionally, analysis was performed using a highly accurate and validated LC-MS/MS methodology which accounted for vitamin D and its metabolites, thus enabling calculation of total vitamin D activity. However, due to the COVID-19 pandemic and subsequent national lockdown restrictions, the analysis was delayed and thus, vitamin D concentrations may have deteriorated during storage. With this is mind, future work may explore how differing storage conditions and durations impact vitamin D retention in pork. Additionally, the present study may be replicated with a scale of varying temperatures and cooking durations for each heat treatment to better understand their respective impact on vitamin D stability. Identical cooking methods could have been considered for each product in a factorial approach however, heat treatments for each respective pork product were selected based on the most common and sensical cooking approaches to allow real-life application and relevance.

## 5. Conclusion

The vitamin $D_3$ and 25(OH)$D_3$ concentration, total vitamin D activity and vitamin D true retention were determined in pork loin chop, mince and sausage after cooking by methods commonly performed in domestic households. Vitamin D concentrations (vitamin $D_3$, 25(OH)$D_3$ and total vitamin D activity) significantly increased after cooking in meat samples, but not in fat samples. Differences in vitamin D concentration were dependent on the pork product rather than the cooking method. Using the adopted cooking protocols, with the exception of sauté mince, all cooking resulted in above 100 % vitamin $D_3$ retention. The true retention differed from 100 % in grill loin, sous-vide loin and pan-fry cooked sausage. Cooking may cause increases in vitamin $D_3$ concentration, but the extent depends on the foodstuff and weight loss.

## CRediT authorship contribution statement

**H.R. Neill:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **C.I.R. Gill:** Conceptualization, Methodology, Writing – review & editing. **E.J. McDonald:** Conceptualization, Writing – review & editing, Funding acquisition. **W.C. McRoberts:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing. **R. Loy:** Methodology, Validation. **L.K. Pourshahidi:** Conceptualization, Methodology, Writing – review & editing, Supervision.

## Declaration of Competing Interest

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

## Data availability

Data will be made available on request.

*Acknowledgements*

This work was funded as part of a Department for the Economy (DfE; Northern Ireland) Co-operative Awards in Science and Technology (CAST) PhD studentship, supported by Devenish Nutrition Limited.

The authors wish to thank Dr David Farrell and Stewart Floyd from AFBI for their respective assistance with the study design and support during analyses.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.133839.

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