# Contents of Cholecalciferol, Ergocalciferol, and Their 25-Hydroxylated Metabolites in Milk Products and Raw Meat and Liver As Determined by HPLC

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The cholecalciferol, ergocalciferol, 25-hydroxycholecalciferol, and 25-hydroxyergocalciferol contents of seven raw meat items and five milk products were analyzed by a high-performance liquid chromatographic (HPLC) method. This included saponification, extraction, purification using one or two semipreparative HPLC steps, and quantification by HPLC. The quantification of cholecalciferol and 25-hydroxycholecalciferol was based on the internal standard (IS) method. Ergocalciferol was used as an IS for cholecalciferol and 25-hydroxyergocalciferol as an IS for 25-hydroxycholecalciferol. The ergocalciferol and 25-hydroxyergocalciferol contents were analyzed using an external standard method. The contents of the vitamin D compounds determined were low; the predominant compounds were cholecalciferol and 25-hydroxycholecalciferol. The cholecalciferol contents ranged in meat samples from <0.05 to 0.48 μg/100 g and in milk product samples from <0.02 to 0.21 μg/100 g. The 25-hydroxycholecalciferol contents were found to be <0.05–0.48 and not detected–0.11 μg/100 g in meat and milk product samples, respectively. Ergocalciferol and 25-hydroxyergocalciferol were not detected or the contents were below or near the limit of determination (0.02–0.05 μg/100 g of fresh weight).

**Keywords:** *Vitamin D; cholecalciferol; 25-hydroxycholecalciferol; ergocalciferol; 25-hydroxyergocalciferol; meat; milk; HPLC; food*

## INTRODUCTION

Vitamin D compounds in meat and milk are either derived from feed or synthesized by the animal; thus, cholecalciferol, ergocalciferol, and their hydroxylated metabolites may theoretically be found in meat and milk products. The number of studies carried out to determine the vitamin D contents of meat and milk products using modern chromatographic methods capable of separating different vitamin D-active compounds is, however, very limited. The contribution of the various vitamin D compounds to the vitamin D activity of these foods can therefore be regarded as poorly known.

The proportions of various vitamin D compounds in meat and meat products are especially poorly known. Koshy and VanDerSlik (1977) were the first to determine the 25-hydroxycholecalciferol contents of bovine liver, kidney, and muscle. Bennink and Ono (1982) and Takeuchi et al. (1984) used high-performance liquid chromatography (HPLC) for determining the cholecalciferol contents of raw and cooked beef and of cattle liver, and Thompson and Plouffe (1993) used HPLC for determining the cholecalciferol and 25-hydroxycholecalciferol contents of meat and fat from farm animals. Mawer and Gomes (1994) used HPLC and/or biospecific methods for determining the vitamin D, 25-hydroxyvitamin D, and 1,25-dihydroxyvitamin $D_3$ contents of raw and cooked chicken and beef. There are no reports on ergocalciferol or its hydroxylated metabolite levels in meat. The occurrence of different vitamin D compounds in milk is better known. All compounds regarded as important were determined using either HPLC or biospecific methods (Takeuchi et al., 1988; Parviainen et al., 1984; Hollis et al., 1981; Reeve et al., 1982).

The aim of the study was to develop a sensitive HPLC method for determining the cholecalciferol and 25-hydroxycholecalciferol contents in milk products and raw meat and liver. A procedure for analyzing ergocalciferol and 25-hydroxyergocalciferol is also described. Using the methods developed, some important representatives of meat and milk products were analyzed for cholecalciferol, ergocalciferol, 25-hydroxycholecalciferol, and 25-hydroxyergocalciferol. The study is part of a comprehensive survey carried out to determine the vitamin D contents in food consumed in Finland (Mattila et al., 1992, 1993, 1994).

## MATERIALS AND METHODS

**Sampling.** Raw meat samples were taken at the end of the grazing season in autumn 1993 (September) and again at the end of the indoor feeding season in spring 1994 (May). The milk product samples were taken in spring 1994 (May) and again in autumn 1994 (October).

The pork liver samples (10 × 200–700 g) were bought from one meat wholesaler in Helsinki. The pork, beef, and beef liver samples (200–600 g) were purchased from 8–10 retail stores representing four major food chains in the Helsinki area. Chicken samples (950–1350 g) were purchased in autumn 1993 from 9 (10 subsamples) and in spring 1994 from 3 (7 subsamples) retail stores. The chickens were halved, and half of each chicken was sampled. Each meat sample was deboned, skinned (chicken), and reduced to 2 × 2 cm cubes; liver samples were reduced to cubes in the same manner. The cubes from the subsamples bought at the same sampling time were pooled (100–150 g of each subsample was taken), homogenized, vacuum-packed in 100-g portions in plastic bags, and stored at −20 °C until analysis.

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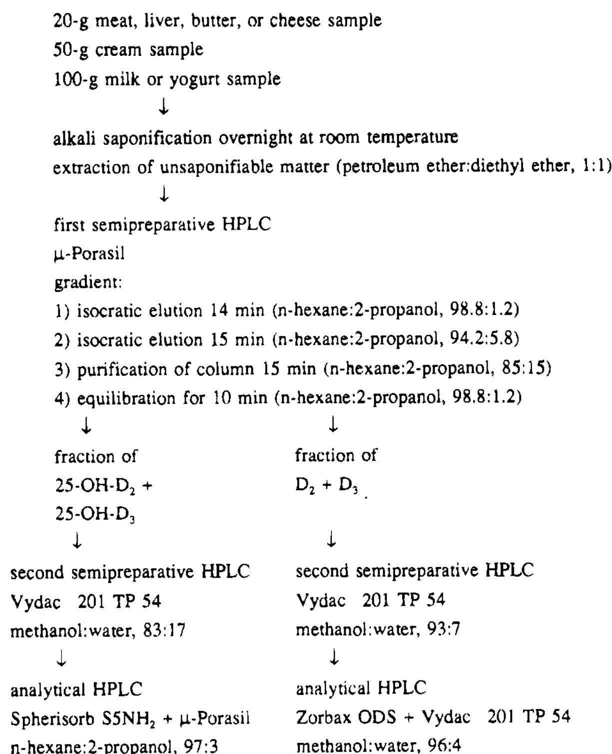


**Figure 1.** Scheme of procedure for determining cholecalciferol, ergocalciferol, and their 25-hydroxylated metabolites in meat and milk product samples.

The milk product samples (0.3–1 kg) were collected from 10 retail stores in the Helsinki area. All 10 subsamples of each item bought at the same sampling time were pooled. Liquid samples (400–500-g aliquots) and butter (100 g of each subsample) were mixed manually. The pooled samples, divided into 100-g (milk, yogurt, and butter) or 50-g (cream) portions, were vacuum-packed in plastic bags (butter) or poured into plastic bottles (milk, yogurt, and cream). The cheese samples were cut into cubes, and 100 g of each subsample was pooled, homogenized, and vacuum-packed in 100-g portions in plastic bags. All samples were stored at −20 °C until analysis.

**Vitamin D Analysis.** *General Principles of the Methods.* The cholecalciferol and 25-hydroxycholecalciferol contents of the pooled raw meat and liver and milk product samples were isolated by alkaline hydrolysis and solvent extraction. The extracts were purified using one or two chromatographic steps. Cholecalciferol and 25-hydroxycholecalciferol were quantified by HPLC using an internal standard (IS) method. Ergocalciferol was used as an IS for cholecalciferol and 25-hydroxyergocalciferol for 25-hydroxycholecalciferol. The quantification was based on peak areas. Blanks, in which no IS was added, were made for all samples. If the blank test showed that the sample contained determinable amounts of ergocalciferol or 25-hydroxyergocalciferol, those compounds were quantified using an external standard method with recovery corrections. For quantification of cholecalciferol and 25-hydroxycholecalciferol by the IS method blank tests were taken into account by subtracting the peak area of the naturally occurring ergocalciferol or 25-hydroxyergocalciferol from the area of the corresponding IS. Determinations were made in triplicate or in dublicate. The purification and quantification scheme is outlined in Figure 1.

*Standards.* The ergocalciferol and cholecalciferol standards were obtained from Sigma Chemical Co., St. Louis, MO. 25-Hydroxycholecalciferol was obtained from Duphar B.V., Vitamins and Chemical Division, The Netherlands, and the 25-hydroxyergocalciferol standard was a generous gift from F. Hoffman-La Roche Ltd., Switzerland. Stock and working standard solutions of the 25-hydroxyergocalciferol and 25-hydroxycholecalciferol standards were made as described earlier (Mattila et al., 1993). The cholecalciferol and ergocalciferol stock solutions were prepared according to the method of Mattila et al. (1992). Working standard solutions were made by diluting 2 mL of the stock solutions in 100 mL of methanol. The concentrations of the working standard solutions were confirmed by measuring the absorbance at 264–265-nm wavelengths and by referring to the known molar absorptivities ($\epsilon$) of 18 840, 18 460, 19 400, and 18 584 for ergocalciferol, cholecalciferol, 25-hydroxyergocalciferol, and 25-hydroxycholecalciferol, respectively. Further dilutions were made to all working standard solutions so that convenient volumes (0.5–2 mL) could be used when the vitamins (24–326 ng) were added to the samples as the IS or for the recovery tests.

*Saponification and Extraction.* Samples were saponified and extracted at room temperature as previously described (Mattila et al., 1992). The sample sizes were 20 (meat, liver, butter, and cheese), 50 (cream), or 100 g (milk and yogurt). Ergocalciferol (24–326 ng) and 25-hydroxyergocalciferol (45–314 ng) were added as IS. The extracts were evaporated, and the residue was dissolved in 1–1.5 mL of *n*-hexane or 0.5% 2-propanol in *n*-hexane and passed through a Millex HV membrane filter (0.45 μm, 1.5 cm, Millipore, France).

*First Semipreparative HPLC Purification.* The first semipreparative cleanup system consisted of a Waters 486 UV detector set at 265 nm, a Waters 600E controller with a pump, a Waters 700 satellite-wisp autosampler, a Waters Millennium chromatography manager, and a μ-Porasil (10 μm, 30 cm × 3.9 mm, Waters) column with a silica guard column. Gradient elution was used (Figure 1). The injection volume was 0.5–1.0 mL of the sample extract, and the flow rate was 1 mL/min. Under these conditions, ergocalciferol and cholecalciferol eluted as one peak in about 10.5–12.5 min. The 25-hydroxyergocalciferol and 25-hydroxycholecalciferol separated, and both compounds eluted in about 24–29 min. The collection time ranged from 1.5 min before to 1.5 min after the retention time of the ergocalciferol and cholecalciferol standard peak and from 2 min before the retention time of the 25-hydroxyergocalciferol standard to 2 min after that of the 25-hydroxycholecalciferol standard. The fractions containing ergocalciferol plus cholecalciferol and 25-hydroxyergocalciferol plus 25-hydroxycholecalciferol were evaporated to dryness under nitrogen and dissolved in 100–200 μL of 7% water in methanol and 100–300 μL of 13% water in methanol, respectively.

*Second HPLC Step for the Ergocalciferol and Cholecalciferol Fraction.* The cholecalciferol content was quantified or the fraction further purified using the HPLC apparatus described above. The column used was a Vydac 201 TP 54 (5 μm, 25 cm × 4.6 mm, The Separations Group) with a $C_{18}$ guard column, and the mobile phase was 7% water in methanol. The flow rate was 1 mL/min, and volumes of 30–180 μL were injected. Under these conditions ergocalciferol and cholecalciferol were separated and eluted in about 16–21 min. If the separation of the ergocalciferol and cholecalciferol peaks from the matrix was not acceptable, the fraction containing ergo- and cholecalciferol was again collected using the collection time of from 2 min before the retention time of ergocalciferol to 2 min after that of the cholecalciferol. After collection, the fraction was evaporated under nitrogen and dissolved in 100 μL of 4% water in methanol.

*Third HPLC Step for the Ergocalciferol and Cholecalciferol Fraction.* When the second semipreparative HPLC purification was needed, cholecalciferol was quantified after the second purification step using two reversed-phase columns, Zorbax ODS and Vydac 201 TP 54, connected with a thin capillary tube. The mobile phase used consisted of 4% water in methanol flowing at a rate of 1 mL/min. The HPLC apparatus was the same as described above. The injection volumes were 50–80 μL.

*Second HPLC Step for the 25-Hydroxyergocalciferol and 25-Hydroxycholecalciferol Fraction.* The 25-hydroxyergocalciferol plus 25-hydroxycholecalciferol fraction from the first semipreparative chromatographic step was further purified by reversed-phase HPLC using an HP 1090 liquid chromatograph with a diode array detector, an HP 9153 C disk drive, and a Vydac 201 TP 54 column with an ODS guard column. The mobile phase contained 83% methanol and 17% water. The

flow rate was 1 mL/min, and volumes of 55–220 μL were injected. The 25-hydroxyergo- and 25-hydroxycholecalciferol separated and eluted in about 16–23 min. The collection from 2 min before the retention time of 25-hydroxycholecalciferol (which eluted first) to 2 min after the retention time of 25-hydroxyergocalciferol was adequate. After the collection, the fraction was evaporated under nitrogen and dissolved in 100–120 μL of *n*-hexane or 0.5% 2-propanol in *n*-hexane.

*Third HPLC Step for the 25-Hydroxyergocalciferol and 25-Hydroxycholecalciferol Fraction.* 25-Hydroxycholecalciferol was quantified using normal-phase HPLC. The apparatus used was the Waters system described above with silica guard and amino columns (Spherisorb S5NH$_2$, PhaseSep, U.K., 5 μm, 25 cm × 4.6 mm, packed in the laboratory) connected with a μ-Porasil column; in some meat samples only the μ-Porasil and guard columns were used. The mobile phase was 2–3% 2-propanol in *n*-hexane. The flow rate was 1 mL/min, and the injection volumes were 35–80 μL.

*Method Reliability Tests.* The reliability of the methods was tested by the recovery and repeatability tests. The recovery tests were made by spiking the samples of meat and milk products with cholecalciferol (40–264 ng) and 25-hydroxycholecalciferol (34–179 ng) before the saponification. Acceptable recoveries of cholecalciferol (94 ± 9.1%, $n = 16$) and 25-hydroxycholecalciferol (94 ± 13%, $n = 17$) were achieved when calculated using the IS. The overall mean recoveries in milk samples were 61 ± 11% ($n = 34$) for ergocalciferol, 53 ± 11% ($n = 8$) for cholecalciferol, 50 ± 12% ($n = 38$) for 25-hydroxyergocalciferol, and 48 ± 13% ($n = 9$) for 25-hydroxycholecalciferol. The corresponding recoveries in meat samples were 69 ± 13% ($n = 48$), 63 ± 14% ($n = 8$), 58 ± 17% ($n = 46$), and 53 ± 17% ($n = 8$), respectively. The repeatability tests were done by monitoring the coefficient of variation (CV%) of the triplicated samples. The mean CV% was 9 ± 7.1% ($n = 14$) for cholecalciferol and 13 ± 10% ($n = 14$) for 25-hydroxycholecalciferol.

The detection limits for ergocalciferol, cholecalciferol, 25-hydroxyergocalciferol, and 25-hydroxycholecalciferol were 0.5 ng/injection. The determination limit was 0.05 μg/100 g for the butter, cheese, and meat samples and 0.02 μg/100 g for the milk, yogurt, and cream samples. The detector responses were linear for ergo- and cholecalciferol and their 25-hydroxylated metabolites in the tested range of 2–300 ng/injection. The coefficient of correlation was >0.999. The response factors were 1.02 for cholecalciferol and 1.05 for 25-hydroxycholecalciferol.

**Moisture Analysis.** The meat samples were analyzed for moisture by drying at 100 ± 2 °C to constant weight (AOAC 952.08, 1990 modified; AOAC, 1990). The moisture in dairy products was determined using IDF methods IDF 80 (1977), IDF 21B (1987, ISO 6731), and IDF 4A (1982, ISO 5534) (IDF, 1977–1987).

**Fat Analysis.** The AOAC method was used to determine the fat contents of the meat samples (AOAC 948.15, 1990; AOAC, 1990). IDF methods used to determine the fat contents of dairy products were IDF 16C (1987), IDF 1C (1987), IDF 5B (1986, ISO 1735), and IDF 80 (1977) (IDF, 1977–1987).

## RESULTS AND DISCUSSION

Owing to the low amounts of cholecalciferol, ergocalciferol, and their 25-hydroxylated metabolites compared with interfering compounds in milk and meat, samples needed to be purified by a number of steps before those compounds could be quantified reliably. In addition, at the analytical HPLC step two connecting HPLC columns were needed to improve the separation. The quantification of 25-hydroxycholecalciferol in milk product samples was especially tedious. On the other hand, use of the various purification steps lowered the overall recoveries. The low overall recoveries were not, however, a problem because use of the IS methods enabled the vitamin losses caused by the purification procedures to be compensated for. The overall recoveries of cholecalciferol and ergocalciferol were equal for every recovery test sample. This observation was made also for 25-hydrocholecalciferol and 25-hydroxyergocalciferol. Good recoveries (94%) were achieved for cholecalciferol and 25-hydroxycholecalciferol when the recoveries were calculated using the IS.

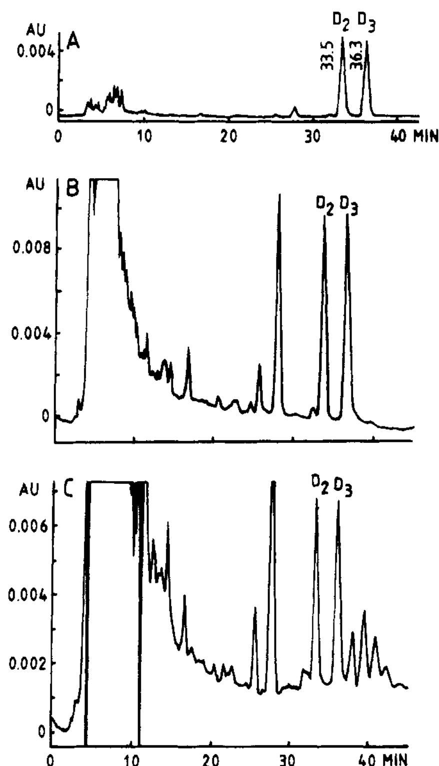


**Figure 2.** Analytical HPLC chromatograms of (A) standard mixture of ergocalciferol (D$_2$) and cholecalciferol (D$_3$), (B) D$_2$ (IS) and D$_3$ in chicken sample, and (C) D$_2$ (IS) and D$_3$ in pork liver sample. Columns, Zorbax ODS + Vydac 201 TP 54; mobile phase, 4% water in methanol; flow rate, 1 mL/min; wavelength, 264 nm.

Examples of the analytical chromatograms of the meat and milk samples are presented in Figures 2–4. Because of the long chromatographic runs, the retention times of the vitamin D compounds changed slightly during the day, especially when a normal-phase HPLC system was used (Figure 4C,D). To ensure reliable peak identification, the standard solution was injected after every sample injection. There was no carry-through effect interfering with the determinations. Because of the many purification steps and long chromatographic runs, it was possible to quantify only two or three samples in triplicate in a week if all of the compounds were determined.

Since the 25-hydroxylated metabolites are more polar than ergo- and cholecalciferol, two methods of dissolving the fractions containing 25-hydroxyergocalciferol and 25-hydroxycholecalciferol before normal-phase HPLC were tested to confirm their solubility: dissolving in n-hexane (as normally done for ergo- and cholecalciferol) or in 2-propanol/*n*-hexane (0.5:99.5). When the latter solvent for milk samples was used, interfering compounds eluted at the same retention time as 25-hydroxycholecalciferol, making reliable quantification impossible. Milk sample fractions were therefore preferably dissolved in *n*-hexane. For meat samples the solvent containing 2-propanol could be used to assure complete solubility of the 25-hydroxy metabolites.

In this study cholecalciferol, ergocalciferol, and their 25-hydroxylated metabolites were determined from milk

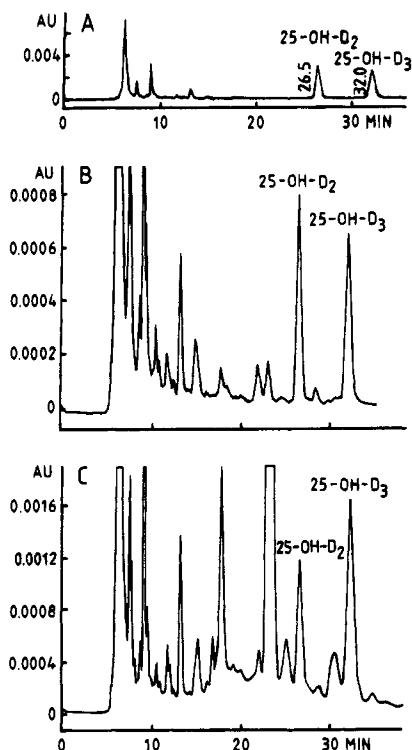


**Figure 3.** Analytical HPLC chromatograms of (A) standard mixture of 25-hydroxyergocalciferol (25-OH-$D_2$) and 25-hydroxycholecalciferol (25-OH-$D_3$), (B) 25-OH-$D_2$ (IS) and 25-OH-$D_3$ in chicken sample, and (C) 25-OH-$D_2$ (IS) and 25-OH-$D_3$ in pork liver sample. Columns, Spherisorb S5$NH_2$ + $\mu$-Porasil; mobile phase, 3% 2-propanol in $n$-hexane, flow rate, 1 mL/min; wavelength, 264 nm.

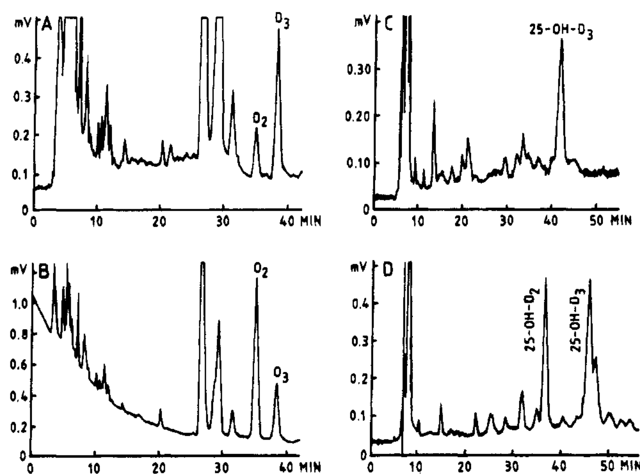


**Figure 4.** Analytical HPLC chromatograms of cream sample: (A) ergocalciferol ($D_2$) and cholecalciferol ($D_3$), $D_2$ not added as internal standard (IS); (B) $D_2$ and $D_3$ + added IS ($D_2$); (C) 25-hydroxycholecalciferol (25-OH-$D_3$), 25-hydroxyergocalciferol (25-OH-$D_2$) not added as IS; (D) 25-OH-$D_2$ (IS) and 25-OH-$D_3$. Analytical conditions for (A) and (B): Zorbax ODS + Vydac 201 TP 54 columns, 4% water in methanol as mobile phase, flow rate 1 mL/min. Analytical conditions for (B) and (C): Spherisorb S5$NH_2$ + $\mu$-Porasil columns, 2% 2-propanol in $n$-hexane as mobile phase, flow rate 1 mL/min, wavelength 264 nm.

products and from raw meat and liver. In addition to these compounds, dihydroxylated metabolites of cholecalciferol and ergocalciferol can also potentially affect the vitamin D activity of milk and meat products. In previous studies, however, the contribution of these dihydroxylated metabolites has been found to play a minor role in the vitamin D activity of the foods above (Hollis et al., 1981; Reeve et al., 1982; Mawer and Gomes, 1994). Thus, the determined compounds give a quite good estimate of milk and meat products as a source of vitamin D.

**Meat and Liver Samples.** The cholecalciferol, ergocalciferol, 25-hydroxycholecalciferol, and 25-hydroxyergocalciferol contents of the raw meat and liver samples were low (Table 1). The most important compounds were cholecalciferol and 25-hydroxycholecalciferol; ergocalciferol and 25-hydroxyergocalciferol were not detected or the contents were below the determination limit (0.05 $\mu$g/100 g). Only in the beef liver sample (spring) was a determinable amount of 25-hydroxyergocalciferol (0.17 $\mu$g/100 g) found.

The cholecalciferol and 25-hydroxycholecalciferol contents of meat and liver samples ranged from <0.05 (all beef samples) to 0.48 (pork liver) $\mu$g/100 g of fresh weight and from 0.05 (beef chuck) to 0.48 (pork liver) $\mu$g/100 g of fresh weight, respectively. It is widely accepted that 25-hydroxycholecalciferol is biologically 5 times as active as cholecalciferol (Reeve et al., 1982), suggesting that the significance of 25-hydroxycholecalciferol to the vitamin D activity of meat was in most cases even more important than the significance of cholecalciferol. Mawer and Gomes (1994) also reported the importance of 25-hydroxyvitamin D in meat samples. On the other hand, Thompson and Plouffe (1993) concluded that residues of 25-hydroxycholecalciferol were of lesser practical significance. The detection limit of their method was, however, fairly high: close to 0.2–1.0 $\mu$g/100 g for cholecalciferol. The detection limit for 25-hydroxycholecalciferol was not mentioned, but if it was of the same magnitude, it may have influenced their conclusion on the importance of 25-hydroxycholecalciferol for the total vitamin D activity in meat.

Because vitamin D compounds are quite stable during cooking (Mawer and Gomes, 1994; Bennink and Ono, 1982), it was possible to evaluate meat and liver as sources of vitamin D, although the determinations were made from raw samples. As shown in Table 1, the best sources of vitamin D from meat and liver samples were pork and beef liver and chicken containing 62–104 IU of vitamin D/100 g (when the total vitamin D content was calculated by assuming that 25-hydroxychole- and ergocalciferol are 5 times as active as cholecalciferol). Pork and beef were quite poor sources of vitamin D. Only minor variations in the results of the two seasons were found. In the case of pork samples (fillet and Boston butt) there was a positive correlation between the fat and cholecalciferol contents. For beef samples this type of correlation was not established due to the undeterminable contents of cholecalciferol. No correlations between the fat and 25-hydroxycholecalciferol contents were found.

Comparison of the present results with previous knowledge is difficult due to differences in methods and in the vitamin D compounds determined. In addition, the sampling was not accurately described in most cases. In those few previous studies available, the results of vitamin D contents in meat varied considerably. Sondergaard and Leerbeck (1982) determined vitamin D contents in meat products using a biological method and reported that pork and beef contained 0.4–0.9 $\mu$g of vitamin D/100 g (calculated as cholecalciferol), agreeing well with the present study. On the other hand, beef and pork liver contained about 10-fold less vitamin D than determined in the present study. Different figures are found in the composition table of Holland et al. (1991): pork, beef, and chicken contained

**Table 1. Cholecalciferol, Ergocalciferol, 25-Hydroxycholecalciferol, and 25-Hydroxyergocalciferol Contents of Meat Products (Micrograms per 100 g of Fresh Weight)**

| item | a | fat (%) | moisture | $D_3$ | $D_2$ | 25-OH-$D_3$ | 25-OH-$D_2$ |
|---|---|---|---|---|---|---|---|
| pork, fillet | S | 7.1 | 70.1 | 0.131 ± 0.0055 | <0.05 | 0.069 ± 0.0042 | ND[b] |
| | A | 6.0 | 71.8 | 0.086 ± 0.0043 | <0.05 | 0.052 | ND |
| | $\bar{x}$ | 6.6 | 71.0 | 0.11 | <0.05 | <0.06 | ND |
| pork, Boston butt | S | 13.5 | 68.2 | 0.29 ± 0.015 | <0.05 | 0.068 ± 0.0040 | ND |
| | A | 14.4 | 67.2 | 0.39 ± 0.076 | <0.05 | 0.069 ± 0.0065 | ND |
| | $\bar{x}$ | 14.0 | 67.7 | 0.34 | <0.05 | 0.07 | ND |
| beef, steak | S | 2.6 | 73.9 | <0.05 | <0.05 | 0.084 | <0.05 |
| | A | 1.8 | 73.8 | <0.05 | ND | 0.085 ± 0.0038 | ND |
| | $\bar{x}$ | 2.2 | 73.9 | <0.05 | <0.05 | 0.08 | <0.05 |
| beef, chuck | S | 9.7 | 69.5 | <0.05 | <0.05 | <0.05 | <0.05 |
| | A | 10.5 | 69.0 | <0.05 | <0.05 | 0.101 ± 0.0036 | ND |
| | $\bar{x}$ | 10.1 | 69.3 | <0.05 | <0.05 | 0.05 | <0.05 |
| pork, liver | S | 2.5 | 73.6 | 0.312 ± 0.0053 | <0.05 | 0.48 ± 0.042 | ND |
| | A | 3.3 | 72.5 | 0.48 ± 0.030 | <0.05 | 0.39 ± 0.089 | ND |
| | $\bar{x}$ | 2.9 | 73.1 | 0.40 | <0.05 | 0.44 | ND |
| beef, liver | S | 2.7 | 70.4 | <0.05 | <0.05 | 0.35 | 0.17 |
| | A | 4.4 | 69.9 | <0.05 | <0.05 | 0.32 ± 0.048 | <0.05 |
| | $\bar{x}$ | 3.6 | 70.2 | <0.05 | <0.05 | 0.34 | 0.09 |
| chicken | S | 10.0 | 69.7 | 0.284 ± 0.0045 | <0.05 | 0.20 ± 0.036 | ND |
| | A | 9.3 | 71.1 | 0.29 ± 0.074 | <0.05 | 0.29 ± 0.012 | ND |
| | $\bar{x}$ | 9.7 | 70.4 | 0.29 | <0.05 | 0.25 | ND |

[a] S, spring; A, autumn; $\bar{x}$, mean. [b] Not detected.

**Table 2. Cholecalciferol, Ergocalciferol, 25-Hydroxycholecalciferol, and 25-Hydroxyergocalciferol Contents of Milk Products (Micrograms per 100 g of Fresh Weight)**

| item | a | fat (%) | moisture (%) | $D_3$ | $D_2$ | 25-OH-$D_3$ | 25-OH-$D_2$ |
|---|---|---|---|---|---|---|---|
| milk | S | 4.0 | 87.4 | <0.02 | <0.02 | <0.02 | ND[b] |
| | A | 3.9 | 87.3 | <0.02 | <0.02 | <0.02 | ND |
| | $\bar{x}$ | 4.0 | 87.4 | <0.02 | <0.02 | <0.02 | ND |
| yogurt, plain | S | 2.6 | 86.7 | <0.02 | <0.02 | ND | ND |
| | A | 2.5 | 87.3 | <0.02 | <0.02 | ND | ND |
| | $\bar{x}$ | 2.6 | 87.0 | <0.02 | <0.02 | ND | ND |
| whipping cream | S | 35.9 | 56.3 | 0.069 ± 0.0029 | <0.02 | 0.069 ± 0.0086 | ND |
| | A | 36.2 | 57.4 | 0.08 ± 0.011 | 0.028 | 0.11 ± 0.041 | ND |
| | $\bar{x}$ | 36.1 | 56.9 | 0.07 | 0.01 | 0.09 | ND |
| cheese, Edam type | S | 24.3 | 42.1 | 0.058 ± 0.0019 | <0.05 | <0.05 | ND |
| | A | 24.1 | 42.6 | 0.153 ± 0.0093 | <0.05 | 0.097 ± 0.0081 | ND |
| | $\bar{x}$ | 24.2 | 42.4 | 0.11 | <0.05 | 0.05 | ND |
| butter | S | 80.6 | 16.7 | 0.18 ± 0.023 | 0.05 | <0.05 | ND |
| | A | 80.6 | 16.6 | 0.21 ± 0.020 | 0.055 ± 0.0052 | 0.10 ± 0.027 | ND |
| | $\bar{x}$ | 80.6 | 16.7 | 0.20 | 0.05 | 0.05 | ND |

[a] S, spring; A, autumn; $\bar{x}$, mean. [b] Not detected.

only traces of vitamin D, while pork and beef liver contained higher amounts. Mawer and Gomes (1994) determined the presence of different vitamin D compounds in the chicken and beef samples using HPLC and/or biospecific methods. They found undeterminable contents of chole- and ergocalciferol, whereas the contents of 25-hydroxyvitamin D ranged from 0.32 to 0.42 μg/100 g. These results are partly different from those obtained in the present study. There are many reasons possibly affecting the variation in vitamin D contents found in different studies, e.g. differences in vitamin D contents in feed and in the exposure of the animals to sunlight.

**Milk and Milk Product Samples.** The levels of vitamin D compounds in milk and milk product samples were also low (Table 2). The predominant compounds were cholecalciferol and 25-hydroxycholecalciferol; 25-hydroxyergocalciferol was not detected in milk and milk product samples. Determinable amounts of cholecalciferol and 25-hydroxycholecalciferol were, however, found only in samples of cream, butter, and cheese. The cholecalciferol and 25-hydroxycholecalciferol levels ranged in these samples from 0.058 (cheese, spring) to 0.21 (butter, autumn) μg/100 g and from <0.05 (cheese and butter, spring) to 0.11 (cream, autumn) μg/100 g, respectively. As in meat, the 25-hydroxycholecalciferol in milk base samples also contributed to the vitamin D activity, a finding also reported by Takeuchi et al. (1988), Hollis et al. (1981), and Kunz et al. (1984).

There was a low seasonal variation in results in the milk base samples; in autumn the contents of the compounds determined were slightly higher than in spring. Sondergaard and Leerbeck (1982) also found seasonal variation in the vitamin D contents of the butter samples.

As in the meat samples, variations in compounds analyzed in previous studies make comparison of their results for milk and milk products with those obtained in this study difficult. Various estimates of the biological activities of vitamin D metabolites have also been used in calculating the total vitamin D contents (Reeve et al., 1982; Hollis et al., 1981). However, Parviainen

et al. (1984), Reeve et al. (1982), Takeuchi et al. (1988), and Hollis et al. (1981) obtained quite similar results for the compounds analyzed in milk in the present study. In previous studies very low contents of cholecalciferol (or of cholecalciferol plus ergocalciferol) and 25-hydroxycholecalciferol (or of 25-hydroxycholecalciferol plus 25-hydroxyergocalciferol) were found (0.004–0.04 and 0.015–0.03 μg/100 g, respectively); other milk products have been less frequently studied. Figures for yogurt, cream, cheese, and butter are mainly found only in composition tables: 0.008–0.04, 0.22–2.00, 0.19–0.35, and 0.3–2.5 μg/100 g, respectively (calculated as cholecalciferol; Holland et al., 1991; Souci et al., 1986; Sondergaard and Leerbeck, 1982). The magnitude of these contents is close to the contents obtained in the present study.

**Conclusions.** The methods developed were well suited for determining the contents of cholecalciferol, ergocalciferol, and their 25-hydroxylated metabolites in milk products and in raw meat and liver. The contents of total vitamin D (as calculated from the compounds determined) were highest in raw pork and beef liver and in chicken. The significance of 25-hydroxycholecalciferol to the total vitamin D activity of meat and milk products is important.

ACKNOWLEDGMENT

We thank F. Hoffman-La Roche Ltd. for donating the 25-hydroxyergocalciferol standard and Kaisa Lehikoinen, Kirsti Risunen, and Jorma Löytynoja for their technical assistance.

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Received for review February 17, 1995. Revised manuscript received May 22, 1995. Accepted June 5, 1995.[⊗] This study was financially supported by the Academy of Finland.

JF9501047

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1995.