

Detection of γ -irradiated raw-milk Camembert cheeses by capillary gas chromatographic analysis of volatile hydrocarbons

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(Received 6 October 1993; revised version received 8 November 1993; accepted 5 January 1994)

A method to detect the irradiation of raw-milk Camembert cheeses by gas chromatographic analysis of radio-induced volatile hydrocarbons, formed by radiolysis of myristic, palmitic and stearic acids, is proposed. The presence, in the sample, of tridecane, 1-dodecene, 1-tetradecene and 1-hexadecane, in association with an increase in levels of pentadecane and heptadecane, can be considered an indication of irradiation. Moreover, the quantity of radio-induced volatile hydrocarbons, formed from the same fatty acid precursor, is proportional to the fatty acid composition in the sample and the 1-alkene/alkane ratio remains constant (~ 1.3) and independent of the fatty acid precursor. In spite of the hydrocarbon stability during ripening and storage and the good linear correlation between the hydrocarbon concentration and the irradiation dose (up to 3.97 kGy), the determination of the latter is approximate because of the experimental error ($\pm 20\%$) and the possible fluctuations of certain parameters of the ionizing treatment (e.g. temperature and dose rate).

INTRODUCTION

The irradiation of raw-milk Camembert cheeses at doses from 2.25 to 3.50 kGy has recently been authorized in France (Anon., 1993). This guarantees the absence of pathogenic bacteria (especially *Listeria monocytogenes*) and reduces the microbial charge considered excessive by certain importing countries.

Among the different irradiation detection methods recommended by the Community Bureau of Reference (Raffi *et al.*, 1993), only the chemical method based on the analysis of volatile radio-induced hydrocarbons has a relevant application to this type of foodstuff, which is rich in fats. The formation of volatile hydrocarbons resulting from the rupture of fatty acid chains is not in itself specific to irradiation. These chemical components can also form after heating or simply as a result of oxidation. Nevertheless, some of these hydrocarbons seem to appear essentially during irradiation. A fatty acid with a general formula $C_m:n$ gives rise mainly to long-chain hydrocarbons (α and β cleavages) with formulae $C_{m-1}:n$ and $C_{m-2}:n+1$ (Nawar, 1986). The demonstration of a characteristic pattern of volatile hydrocarbons in some fatty foods can, in these circumstances, be used as an irradiation detection method (Nawar & Balboni, 1970; Nawar, 1988).

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This method has been used essentially, and often with success, for detecting irradiation of meat and meat products (Biedermann *et al.*, 1989; Meier and Biedermann, 1990; Ammon *et al.*, 1992; Morehouse *et al.*, 1993; Singh *et al.*, 1993; Lesgards *et al.*, 1993; Sjoberg *et al.*, 1992) or of seafoods (Biedermann *et al.*, 1992; Morehouse & Ku, 1992). The aim of the present work is to see whether this method can also be applied to full-cream milk products and especially to raw-milk Camembert cheeses.

MATERIALS AND METHODS

Test materials

Raw-milk Camembert cheeses (45% fat) were purchased from the Union Coopérative Laitière (UCL) of Isigny (France). After 15 days of ripening at 10°C in the cheese manufacture, the samples were wrapped and γ -irradiated (⁶⁰Co irradiator (Sultzer, Winterthur, Switzerland)); activity 3.6×10^5 Ci; dose rate 1 kGy/h, temperature: 4°C) at different doses (0, 1.20 kGy, 1.91 kGy and 3.97 kGy). Dosimetry was realized with Far West Technology dosimeters (Goleta, USA), calibrated against a reference alanin dosimeter. The dose heterogeneity was $\pm 10\%$.

The ripening was carried out for 5 days more in the manufacture. At that time, the cheeses (reference and irradiated samples) were transported by lorry (refrigerated at 4°C) the same day to the authors' laboratory and ripened again for a second period of 11 days in an oven at 10°C, then stored for 43 days at 4°C.

Chemicals

All solvents and chemicals used were of the highest purity available. UV grade *n*-pentane and isooctane were obtained from Merck (Darmstadt, Germany) and 2-propanol from Carlo Erba (Milano, Italy). They did not contain any traces of hydrocarbons (tested by gas chromatography (GC)) and were not redistilled before use. Anhydrous sodium sulphate was obtained from Prolabo (Paris, France) and florisil from Merck (Darmstadt, Germany). The *n*-alkanes (tridecane, pentadecane, heptadecane, eicosane) and 1-alkenes (dodecene, tetradecene, hexadecene) used as standards were obtained from Sigma (St Louis, MO, USA). The fatty methyl ester standards were also obtained from Sigma.

Ultrapure water was obtained from a Milli Q plus apparatus (Millipore, Bedford, USA).

Extraction of fat and isolation of volatiles

The method used was the method proposed by Schulzki *et al.* (1992) with minor modifications.

Samples of cheese (50 g), cut into small pieces, were put in a centrifuge glass bottle and melted in a water bath for 30 min at 50°C. Pentane/isopropanol (50 ml; 3:2 v/v) were gradually added with gentle shaking. The mixture was centrifuged (Beckman, J2-21) for 10 min at 900 × *g*. The upper pentane layer was removed, put in a flask and evaporated in a rotary evaporator (water bath at 50°C) to a volume of about 5 ml. Approximately 5 g of sodium sulphate (heated to 650°C for 5 h before use) were added. The mixture was shaken gently; the flask was stoppered and left at room temperature for 1 h. The solidified fat was remelted in an oven (50°C), then filtered through a paper. The flask and the paper were rinsed carefully with pentane. The solvent was then totally evaporated in a rotary evaporator (water bath at 50°C).

A 1 g sample of the extracted fat was dissolved in pentane (1 ml) containing the internal standard (eicosane). The mixture was applied to the florisil column prepared as follows: florisil was first placed in a furnace at 550°C for 5 h, then cooled in a desiccator and poured into a stoppered flask; ultra-pure water (3%) was added; the flask was stoppered and shaken until no lumps could be seen and the powder flowed freely. The mixture was left at room temperature to equilibrate overnight; the partially deactivated florisil was suspended in pentane and packed to a height of 10 cm into a 20 mm i.d. glass column (approx. 20 cm high); anhydrous sodium sulphate (1 cm high) was added.

The hydrocarbons were eluted with 60 ml of pen-

tane. The eluant was evaporated in a rotary evaporator (water bath at 45°C) without vacuum to a volume of about 5 ml. Isooctane (0.8 ml) was added and the remaining pentane was evaporated as above. The residue (hydrocarbons dissolved in isooctane) was diluted to 1 ml with isooctane in a volumetric flask and stored at 4°C.

Gas chromatography of hydrocarbons

The hydrocarbons were analyzed by using a gas chromatograph (Varian 3300, Sunnyvale, USA) fitted with a HP-FFAP capillary column (25 m × 0.20 mm i.d., 0.33 μm film thickness (Hewlett-Packard, Geneva, Switzerland)) and equipped with a flame ionization detector (250°C). Operating conditions were as follows: injector 230°C, column temperature programme: 50°C (2 min) followed by a 4°C/min increase to 200°C; the final temperature was held for 10 min. Nitrogen was used as a carrier gas. A 1 μl aliquot of the isooctane solution was injected into the gas chromatograph operating in the splitless mode.

The concentrations of the hydrocarbons were obtained by using an internal standard (eicosane).

Triplicate analyses were carried out on reference and irradiated (at 1.20, 1.91 and 3.97 kGy) samples during the second phase of ripening (three determinations) and the storage duration (three determinations).

The recovery rates for the various hydrocarbons were obtained by adding known concentrations of hydrocarbon standards to the extracted fat dissolved in pentane. The hydrocarbon concentrations in these spiked samples were determined by the procedure described above. The recovery rate correction factors of the method were calculated for each hydrocarbon according to the following formula:

$$K = \frac{A(C_{m:n})}{Q(C_{m:n})} \times \frac{Q(C_{20:0})}{A(C_{20:0})}$$

where $Q(C_{m:n})$ and $Q(C_{20:0})$ were, respectively, the quantities of hydrocarbon and internal standard in the sample and $A(C_{m:n})$ and $A(C_{20:0})$ the areas of the peaks of the two compounds.

Fatty acid analysis

The fatty acid methyl esters were prepared according to the ISO standard method (ISO, 1978). They were quantitated by gas chromatography using the same apparatus and column as for the hydrocarbon analysis with following temperature programme: 70°C (2 min) followed by a 8°C/min increase to 230°C; the final temperature was held for 10 min.

RESULTS AND DISCUSSION

Camembert cheese fat (24.2 g/100 g of cheese), contains large amounts of myristic, palmitic, stearic and oleic acids (82.9% of the determined fatty acids). Their con-

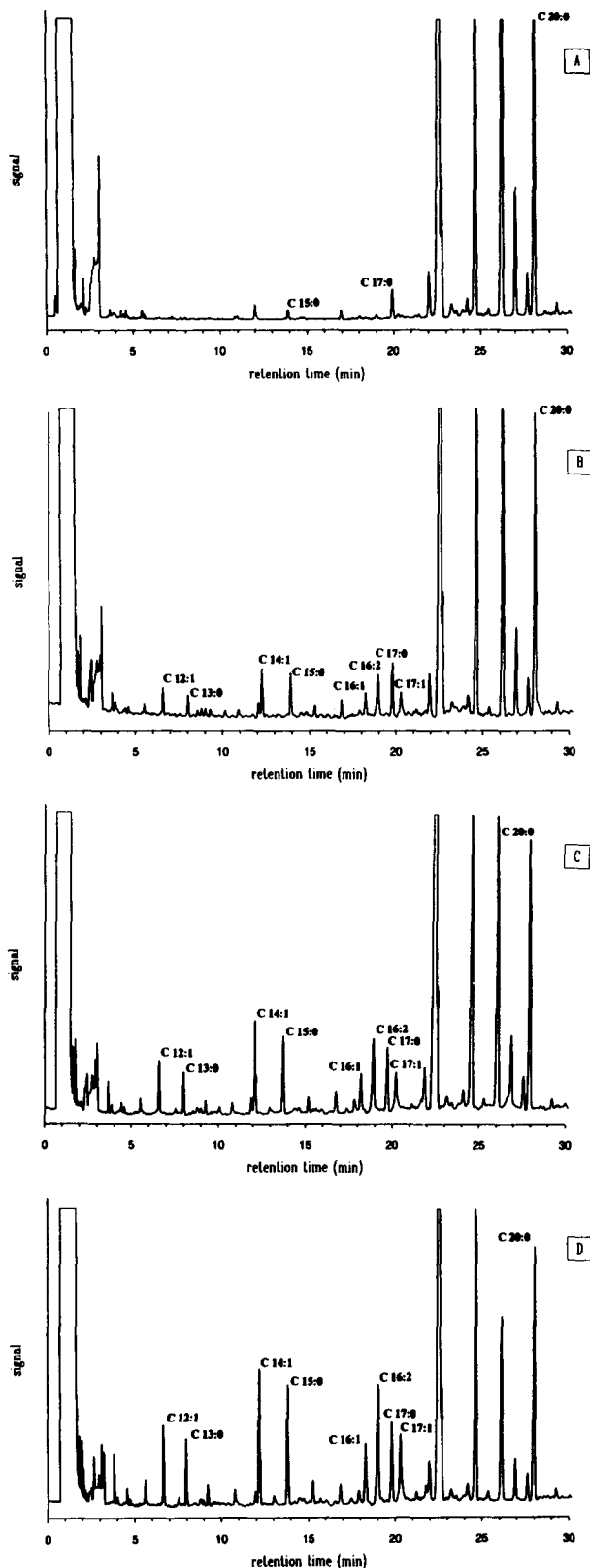


Fig. 1. Gas chromatographic analyses of volatile hydrocarbons present in (A) unirradiated and irradiated ((B) 1.21 kGy, (C) 1.91 kGy and (D) 3.97 kGy) Camembert cheeses after 21 days of ripening (e.g. 6 days after irradiation).

centrations (respectively, 0.114, 0.289, 0.115 and 0.228 mg/mg fat) are in agreement with those published elsewhere (Souci *et al.*, 1989). Therefore, when Camembert cheeses are irradiated, one would expect to find the hydrocarbons formed by decarboxylation of these fatty

Table 1. Correction factor for recovery (\pm SD) of the various hydrocarbons analyzed in Camembert cheese samples^a

Hydrocarbons	K values
C _{12:1}	1.29 \pm 0.10
C _{13:0}	1.51 \pm 0.13
C _{14:1}	1.48 \pm 0.11
C _{15:0}	1.56 \pm 0.11
C _{16:1}	1.63 \pm 0.17
C _{17:0}	1.50 \pm 0.12
C _{20:0}	1.00

^a Arbitrary value 1.00 for the internal standards all the determinations were carried out in triplicate.

acids, mainly tridecane (C_{13:0}) and 1-dodecene (C_{12:1}) from myristic acid (C_{14:0}), pentadecane (C_{15:0}) and 1-tetradecene (C_{14:1}) from palmitic acid (C_{16:0}), heptadecane (C_{17:0}) and 1-hexadecene (C_{16:1}) from stearic acid (C_{18:0}), 8-heptadecene (C_{17:1}) and 1,8-hexadecadiene (C_{16:2}) from oleic acid (C_{18:1}), according to the theory of Nawar (1986).

Two of these hydrocarbons (pentadecane and heptadecane) were detected in the non-irradiated samples, at very low concentrations for pentadecane (Fig. 1(A)). For the chromatograms obtained with the irradiated samples, the eight hydrocarbons mentioned above are always present in noticeable quantities, even at the lowest dose (Figs 1(B)–(D)). The identities of the various peaks have been confirmed by comparison of the retention times (very consistent from an experiment to another) with known standards. Unfortunately, two of these standards (8-heptadecene and 1,8-hexadecadiene) are not commercially available and their eventual use as evidence of an ionizing treatment cannot be recommended. For this reason, in the follow-up to this work, only the six hydrocarbons formed from myristic, palmitic and stearic acids have been studied since their identification can be carried out easily and without ambiguity.

Four of these hydrocarbons (tridecane and 1-dodecene, 1-tetradecene, 1-hexadecene) only seem to be present in the irradiated samples. Their formation in Camembert cheese, in association with an increase in concentration of pentadecane and heptadecane, could indicate an irradiation. In order to confirm this possibility, these three pairs of hydrocarbons were quantitated in control and irradiated Camembert cheeses during ripening and then during prolonged storage at $\pm 4^\circ\text{C}$.

The measured values of the recovery rate correction factors for each hydrocarbon are indicated in Table 1 and the obtained results concerning the amounts of hydrocarbons in the different samples are given in Table 2. No hydrocarbon formation is observed in the non-irradiated samples, neither in the second phase of ripening (e.g. after the date of irradiation) nor during storage. The levels of pre-existing hydrocarbons (pentadecane and heptadecane) stay remarkably constant. The same result is obtained in the irradiated samples: the hydrocarbon concentration does not vary during storage. Merritt *et al.* (1978) and Morehouse and Ku (1992) had already noticed the high stability of radio-

Table 2. Evolution of the amount of hydrocarbons found in γ -irradiated and non-irradiated control Camembert cheeses during the second phase of ripening (after irradiation) at 10°C (17 days) then storage (43 days) at 4°C^a

Dose (kGy)	Days after irradiation	nmol of hydrocarbons/g fat					
		C _{12:1}	C _{13:0}	C _{14:0}	C _{15:0}	C _{16:1}	C _{17:0}
0	6	0.00	0.00	0.00	0.42	0.00	1.09
	10	0.00	0.00	0.00	0.41	0.00	1.05
	13	0.00	0.00	0.00	0.43	0.00	1.17
	24	0.00	0.00	0.00	0.41	0.00	1.20
	39	0.00	0.00	0.00	0.39	0.00	1.18
	60	0.00	0.00	0.00	0.40	0.00	1.25
1.20	6	1.40	0.98	2.49	2.03	0.98	2.00
	10	1.14	0.89	2.31	2.01	1.00	2.08
	13	1.21	0.82	2.06	1.77	0.88	1.53
	24	1.39	1.08	2.56	2.16	0.99	1.95
	39	1.37	1.04	2.59	2.17	1.04	1.88
	60	1.36	1.05	2.44	2.21	0.98	1.85
1.91	6	2.41	1.74	4.22	3.45	1.83	2.97
	10	2.59	1.92	4.67	3.76	1.78	2.54
	13	2.24	1.56	3.82	3.13	1.56	2.25
	24	2.34	1.80	4.20	3.58	1.75	2.70
	39	2.07	1.63	3.74	3.30	1.50	2.25
	60	2.16	1.88	4.12	—	1.76	2.83
3.97	6	5.10	3.90	8.34	7.52	3.64	3.48
	10	4.55	3.22	7.73	6.20	3.12	3.37
	13	4.95	3.05	8.12	6.53	2.96	3.21
	24	4.42	3.44	7.59	6.62	3.05	—
	39	4.25	3.23	7.38	6.20	3.00	3.30
	60	4.46	3.64	7.78	7.35	3.35	3.77

^a The hydrocarbon concentration has an experimental error of approximately $\pm 15\%$; all the determinations were carried out in triplicate.

induced hydrocarbons, respectively, in irradiated beef and shrimps) or during ripening. The variations observed can be explained by inaccurate measurements (experimental error of approximately $\pm 15\%$). This result shows that the increase in hydrocarbon concentration, observed as the irradiation dose increases, is only due to the irradiation treatment.

Assuming, as has just been demonstrated, that the hydrocarbon concentration remains stable during ripening and storage, it is possible to determine the average concentration of each radio-induced hydrocarbon (expressed in nmols/mmol of fatty acid precursor) relative to the radiation dose (Table 3). The experimental error in the determination (which takes into account the experimental error in the determination of the fatty acid composition, estimated at $\pm 5\%$) is in the order of $\pm 20\%$ for most of the hydrocarbons. However, it is higher in the determination of heptadecane, especially after irradiation at low doses. This is due to the fact that this compound exists naturally, and in important quantities, in the samples analyzed and that it forms in relatively low quantities by irradiation. Nevertheless, it can be demonstrated that, usually, a good linear correlation exists between the hydrocarbon concentration and the irradiation dose (Fig. 2). The correlation rate always exceeds 99.8%, except for heptadecane ($> 98.6\%$). Results indicated in Table 3 show that saturated fatty

Table 3. Mean concentration of radio-induced hydrocarbons found in γ -irradiated Camembert cheese^a

Hydrocarbon	Dose (kGy)	Mean concentration (nmol/mmol fatty acid precursor)	Standard standard deviation	Relative deviation (%)
C _{12:1}	1.20	2.62	0.52	20
	1.91	4.61	0.83	18
	3.97	9.26	1.57	17
C _{13:0}	1.20	1.95	0.68	35
	1.91	3.52	0.70	20
	3.97	6.84	1.23	18
C _{14:1}	1.20	2.14	0.38	18
	1.91	3.66	0.55	15
	3.97	6.94	0.97	14
C _{15:0}	1.20	1.46	0.29	20
	1.91	2.69	0.75	28
	3.97	5.61	0.90	16
C _{16:1}	1.20	2.41	0.46	19
	1.91	4.19	0.75	18
	3.67	7.88	1.50	19
C _{17:0}	1.20	1.79	1.07	60
	1.91	3.54	2.01	57
	3.97	5.62	1.07	19

^a Values extrapolated for an absorbed dose of 1 kGy: experimental error approximately $\pm 20\%$.

acid degradation products with one carbon less (α -cleavage) are less likely than those with two carbon atoms less (β -cleavage). On the other hand, for each irradiation dose, the ratio of radio-induced hydrocarbon concentration $[C_{m-2:n+1}]/[C_{m-1:n}]$ is remarkably constant. This is in the order of 1.3, regardless of the irradiation dose and the chain length of the fatty acid precursor.

From the results in Table 3, it is also possible to calculate the total concentration of radio-induced hydrocarbons $[C_{m-1:n}] + [C_{m-2:n+1}]$ from each fatty acid precursor $C_{m:n}$ for an extrapolated dose of 1 kGy (Table 4). Allowing for the experimental error, this parameter is not dependent on either the irradiation dose (which is normal since a linear relationship exists between the hydrocarbon concentration and the irradiation dose) or on the chain length of the saturated fatty acid precursor. Therefore, the formation of hydrocarbons (alkane + 1-alkene) is approximately proportional to the composition of fatty acids in the cheese samples.

The irradiation detection method based on the analysis of volatile radio-induced hydrocarbons therefore seems well adapted to Camembert cheese. In fact, four hydrocarbons—tridecane, 1-dodecene, 1-tetradecene and 1-hexadecene—formed by radiolysis of myristic, palmitic and stearic acids, never appear in non-irradiated cheeses, either during ripening or during storage. Their presence in a Camembert cheese, in association with an increase in levels of pentadecane and heptadecane can be considered as indicative of an irradiation. This can be confirmed by a more precise analysis of the chromatogram obtained. Indeed, the quantity of volatile hydrocarbons (alkane + 1-alkene) formed from

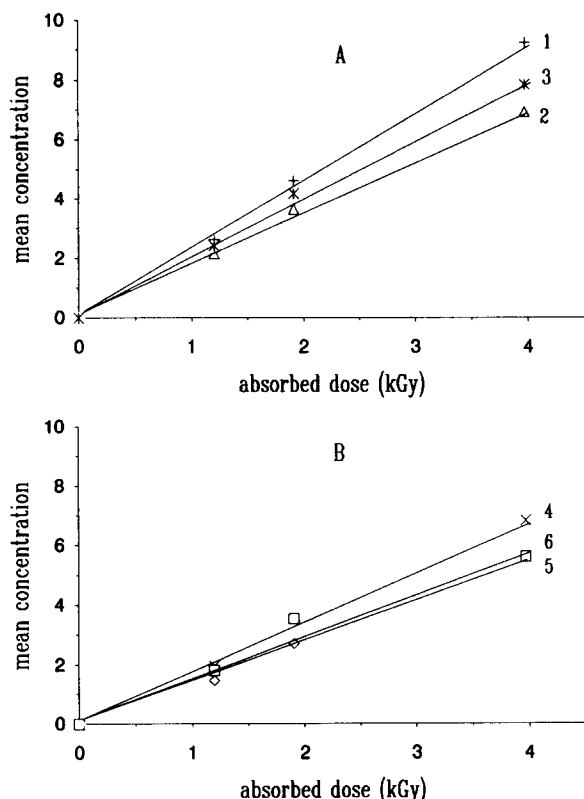


Fig. 2. Mean concentration of radio-induced (A) 1-alkenes and (B) alkanes (expressed in nmol/mmol precursor fatty acid) versus absorbed dose ((1) $C_{12:1}$, (2) $C_{14:1}$, (3) $C_{16:1}$, (4) $C_{13:0}$, (5) $C_{15:0}$, (6) $C_{17:0}$).

the same saturated fatty acid precursor should be proportional to the fatty acid composition in the sample analysed. The 1-alkene to alkane ratio should remain constant (in the order of 1.3) and independent of the saturated fatty acid precursor.

Since the quantity of each radio-induced hydrocarbon increases linearly with the irradiation dose and remains stable during ripening and storage, the determination of the irradiation dose should be possible if a reference Camembert cheese is available, thus allowing a standardization. In fact this determination can only be very approximate. This is partly due to the high degree of experimental error ($\pm 20\%$) encountered in the determination of hydrocarbon concentrations, but also because of the possible ignorance of certain parameters of the ionizing treatment, e.g. the temperature or the dose rate. Indeed, fluctuations in the latter can lead to important variations in volatile hydrocarbon concentrations.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Direction Générale de la Concurrence, de la Consommation et de la Répression des Fraudes of France for financial assistance (Grant no. 93/18/903/527). The authors would also like to thank the Union Coopérative Laitière of Isigny (France) for cheese supplies and irradiation facilities.

Table 4. Mean concentrations of hydrocarbons ($C_{m-1:n}$ and $C_{m-2:n+1}$) issued from a fatty acid precursor $C_{m:n}$ and produced by irradiation in Camembert cheese^a

Fatty acid precursor ($C_{m:n}$)	Doses (kGy)	Hydrocarbon ($C_{m-1:n} + C_{m-2:n+1}$) concentration (nmol/mmol precursor/kGy)
$C_{14:0}$	1.20	3.80 (2.18 + 1.62)
	1.91	4.25 (2.41 + 1.84)
	3.97	4.06 (2.33 + 1.72)
$C_{16:0}$	1.20	3.04 (1.82 + 1.22)
	1.91	3.32 (1.91 + 1.41)
	3.97	3.16 (1.74 + 1.42)
$C_{18:0}$	1.20	3.50 (2.01 + 1.49)
	1.91	4.04 (2.19 + 1.85)
	3.97	3.40 (1.98 + 1.42)

^a For each dose, six determinations realized in triplicate (e.g. 18 determinations) have been taken into account.

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