LWT - Food Science and Technology 224 (2025) 117835

Contents lists available at ScienceDirect

LWT

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

Negative correlation between oil lipolysis and its LC-PUFA content in simulated digestion

Gabriele Beltrame [1], Iida Valta, Annelie Damerau, Kaisa M. Linderborg [*]

Food Sciences, Department of Life Technologies, University of Turku, FI-20014, Turku, Finland

ARTICLE INFO

Keywords:
Simulated digestion
INFOGEST
Lipolysis
Pancreatin
DHA

ABSTRACT

The growing awareness of the pro-oxidant conditions of the gastrointestinal tract promotes attention to the fate of n-3 PUFAs after ingestion. These investigations primarily rely on *in vitro* models, with INFOGEST 2.0 being the most commonly applied. However, scarce attention is currently paid to the hydrolysis degree of the oils. In the present short communication, lipid digesta of DHA-rich oils, obtained after *in vitro* digestion, were analyzed with NMR spectroscopy. Rapeseed oil was used as positive control. Results showed that the DHA content of the oil negatively affected lipolysis, with an observed negative non-linear relationship between lipolysis extent and the long chain n-3/n-6 fatty acid ratio of the oil. ^{13}C NMR revealed a clear negative relationship between lipolysis and relative amount of 22:6n-3 in the *sn*-1,3 position. This intrinsic obstacle should be taken into account during the *in vitro* research on behavior of n-3-rich oils in the gastrointestinal tract.

1. Introduction

Omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs), in particular docosahexaenoic acid (22:6n-3, DHA) and eicosapenaenoic acid (20:5n-3, EPA), play an essential role in the human well-being at all development stages and provide protection against disorders such as cardiovascular diseases, osteoporosis, and cancer (Saini & Keum, 2018). A major issue with dietary n-3 PUFAs is their proneness to oxidation, which decreases sensorial and nutritional quality and even generates potentially toxic compounds. Compared to production and storage, the interest on the behavior of n-3 PUFAs in the physiologically pro-oxidant environment of the gastrointestinal tract has risen in the field of lipid oxidation only recently. This phenomenon is usually studied employing *in vitro* models of the digestion process. Of these, INFOGEST 2.0 is the most recent, and the one with the widest consensus and applications (Brodkorb et al., 2019). INFOGEST 2.0 has been applied to the study of bioaccessibility of lipid compounds (recently reviewed by (Tan et al., 2022)) but most report focused solely on free fatty acids release. The use of INFOGEST in the field of lipid oxidation has been largely limited to the investigation of primary and secondary oxidation products. In addition, due to the complexity of the oxidation reaction, these studies require analysis (Damerau et al., 2020) and sample amounts often different from *in vitro* bioaccessibility studies (Tan et al., 2020).

The difficulty in the hydrolysis of n-3 PUFA esters, especially DHA, is known in the field of lipid restructuring (Halldorsson et al., 2003) but applied conditions differ from those *in vitro* or *in vivo*. While there are indications of easier release of saturated (SAFA) or monounsaturated (MUFA) fatty acids compared to n-3 PUFAs in food formulation studies (Gayoso et al., 2019; Gomes et al., 2019), evidence remains scattered in the field of lipid oxidation. Therefore, also in light of our recent studies on lipid oxidation of n-3 PUFAs during simulated digestion (Beltrame et al., 2023, 2024), the scope of the present short communication was to detail the achievable degree of lipolysis of different oils rich in n-3 LC-PUFA, in particular 20:5n-3 and 22:6n-3, in INFOGEST 2.0 model. The work aimed to verify the effect of fatty acid composition and triacylglycerol (TAG) structure on hydrolysis with the aid of ^{1}H and ^{13}C NMR spectroscopy.

2. Materials and methods

2.1. Samples and reagents

Rapeseed oil, two different oils from *Schizochytrium* sp., cod liver oil (henceforth fish oil), and fish oil concentrate were obtained from

* Corresponding author. Food Sciences Unit, Department of Life Technologies University of Turku, FI-20014, Turku, Finland.
E-mail addresses: gabriele.beltrame@utu.fi (G. Beltrame), iida.a.valta@utu.fi (I. Valta), annelie.damerau@utu.fi (A. Damerau), kaisa.linderborg@utu.fi (K.M. Linderborg).
[1] Present address: Food and Nutrition Science, Department of Life Sciences (LIFE), Chalmers University of Technology, SE-41296 Gothenburg, Sweden.

https://doi.org/10.1016/j.lwt.2025.117835
Received 20 March 2025; Received in revised form 17 April 2025; Accepted 23 April 2025
Available online 23 April 2025

commercial sources. Tridocosahexaenoin was supplied by Larodan (Solna, Sweden).

All solvents and chemicals were supplied by SigmaAldrich (Saint Louis, MO, USA). Salts were supplied by SigmaAldrich, VWR (Leuven, Belgium), Scharlau (Barcelona, Spain), Avantor (Radnor, PA, USA), Fischer Chemicals (Zürich, Switzerland), and Merck (Darmstadt, Germany).

Rabbit gastric lipase (RGE15-500, lipase activity 28.0 ± 6.6 U/mg) was obtained from Lipolytech (Marseille, France). Porcine pepsin, α-amylase, porcine pancreatin (lipase activity 77.8 ± 18.8 U/mg), and bile salts were purchased from SigmaAldrich. Potato flour, whey protein concentrate, and wheat bran were supplied from commercial sources.

2.2. Fatty acid analysis

Fatty acids were analyzed with gas chromatography-flame ionization detector (GC-FID) as methyl esters according to previously published protocol (Damerau et al., 2020).

2.3. Simulated digestion

A defatted simulated meal was prepared for the static *in vitro* digestion experiments following a previously published protocol (Beltrame et al., 2023). Oil and simulated meal were combined prior to digestion.

The simulated *in vitro* digestion was performed according to the INFOGEST 2.0 protocol (Brodkorb et al., 2019). The entire procedure was carried out in dim light, while the digestion itself was carried out in darkness. The compositions of simulated saliva, gastric fluid, and intestinal fluid were as stated in the protocol. Enzyme solutions in their respective simulated fluids were prepared the day of the experiments. The required aliquots of $CaCl_2 \bullet 2\ H_2O$ 0.3 M were added to the simulated fluids only prior to enzyme addition. Solutions were preheated to 37 °C before use. The pH was adjusted with HCl and NaOH 1 M solutions with aliquots verified prior to digestions. The use of shaking during incubation was tested with a separate set of digestions of rapeseed oil.

Simulated oral phase. 0.90 g of simulated meal and about 40 mg of oil were added with 1 mL of simulated saliva fluid, containing α-amylase 75 U/mL. The pH of the mixture was adjusted to 7. Chewing process was simulated with a clean glass rod by randomly hitting the mixture 32 times. Subsequently, mixture was incubated for 2 min at 37 °C.

Simulated gastric phase. The simulated oral bolus was added with 1 mL of simulated gastric fluid containing pepsin and rabbit gastric lipase to reach enzymatic activity of 2000 U/mL and 60 U/mL, respectively (final chyme volume). After pH adjustment to 3, the mixture was incubated for 2 h at 37 °C.

Simulated intestinal phase. The simulated gastric chyme was added with 2 mL of simulated intestinal fluid containing bile salts to reach concentrations in the digesta of 10 mM. The amount of pancreatin used to reach 2000 U/mL in the digesta was according to (Grundy et al., 2021). The effect of the increase of pancreatin concentration from the one used in (Beltrame et al., 2023) was tested with separate digestions of rapeseed oil. After pH adjustment to 7, the mixture was incubated for 2 h at 37 °C.

Lipids were extracted immediately after digestion using hexane:isopropanol 2:1 v/v with BHT 0.05%, according to previously published protocol (Beltrame et al., 2023).

2.4. ^{1}H and ^{13}C NMR spectroscopy

Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were collected from undigested oils and lipids extracted from digesta and recovered with $CDCl_3$ following previously published conditions (Damerau et al., 2020). Tetramethylsilane (TMS) signal at 0.00 ppm was used to normalize the ^{1}H peak integrations. ^{13}C NMR spectra of undigested oils were collected with the same instrument (150.92 MHz) following the semiquantitative parameters published by Aursand (Aursand et al., 2007). NMR data was processed with software TopSpin 4.0.7 (Bruker, MA, USA).

2.5. Statistical analysis and data fitting

The statistical analysis was performed with RStudio (RStudio, 2020) with a confidence level of 95% ($p < 0.05$) for statistical significance. Linear and non-linear relationships between chromatographic and spectroscopic data were calculated with Origin 2016 (OriginLab Corp., Northampton, MA, USA).

3. Results and discussion

3.1. Fatty acid analysis

The fatty acid composition of the oils used in this study was determined before the digestion experiments. The results are reported in Table 1. The oils selected for this study were different from each other, especially in terms of MUFA and PUFA contents.

As first step of the present work, the fatty acid composition of rapeseed oil digestates was measured to verify eventual losses in fatty acids due to the digestion and extraction process. The results are reported in Supplementary Fig. 1b. There were no differences ($p > 0.05$) in the total fatty acid compositions between rapeseed oil and its different digestates. Thus, the protocol reported in Section 2.5.3 extracted and methylated efficiently both free and glycerol-bound fatty acids from the digesta.

3.2. ^{1}H NMR: digestion products and TAG hydrolysis

The degree of hydrolysis of TAGs and the formation of digestion products (di- and monoacylglycerols) was evaluated with ^{1}H NMR using the signal area of the glyceryl backbone. In the proton spectra, the TAG signals were observed at 4.14 ppm, 4.28 ppm, and 5.27 ppm. 1,2-diacylglycerol (1,2-DAG), undistinguished from 2,3- diacylglycerol, signals were observed at 3.73 ppm, 4.32 ppm, and 5.08 ppm. 2-monoacylglycerol (2-MAG) signals were observed at 3.83 ppm and 4.93 ppm. Signals ascribable to 1-monoacylglycerol (1-MAG) were observed as well at 3.65 ppm, 3.94 ppm, and 4.23 ppm (Nieva-Echevarría et al., 2016). Both lipases utilized during simulated digestion have no affinity for position *sn*-2 (Rogalska et al., 1990). Therefore, the presence of 1-MAG was ascribed to acyl migration from position *sn*-2 to position *sn*-1 (Halldorsson et al., 2003).

The scope of the present work was to outline the achievable degree of lipolysis at simulated digestion conditions of oils rich in n-3 LC-PUFA, in particular 20:5n-3 and 22:6n-3. According to the INFOGEST protocol authors (Brodkorb et al., 2019), pancreatin amount in the digesta should be based on its measured trypsin activity. Also, the authors stated that a lipase activity of 2000 U/mL in the chyme should be sought only in case of "high-fat-containing foods or if fat digestion is the aim of the study" (Brodkorb et al., 2019). As our previous work on simulated digestion of lipids utilized a simulated meal with low fat concentration (about 5% w/w) (Beltrame et al., 2023, 2024) and we opted for a protocol unbiased towards lipid digestion, the amount of pancreatin was based on another previously reported, widely utilized protocol (Versantvoort et al., 2005). In the present work, the fat concentration in the simulated meal remained at 5% w/w. The hydrolysis degrees of rapeseed oil obtained with the pancreatin dosage prescribed in (Versantvoort et al., 2005) and the dosage required to reach 2000 U/mL were compared. The effect of shaking was tested as well. The results, expressed as total ^{1}H TAG area, are reported in Supplementary Fig. 1. The increase in pancreatin and use of shaking led to a significant ($p < 0.05$) increase in the hydrolysis of TAGs, i.e. a reduction of ^{1}H TAG area from −20±7% to −65±2% after digestion. Therefore, digestions of the high n-3 LC-PUFA oils were performed with 2000 U/mL pancreatin dosage coupled with shaking. It

Table 1
Main fatty acid composition of tridocosahexaenoin and different oils utilized in this study.

Fatty acid	Rapeseed oil	Fish oil	Microalgae oil1	Microalgae oil2	Fish oil concentrate	Tridocosahexaenoin
	w/w%					
14:0	n.d.	4.09 ± 0.01	9.93 ± 0.03	1.18 ± 0.01	0.49 ± 0.01	n.d.
16:0	4.69 ± 0.03	9.93 ± 0.03	19.76 ± 0.04	19.62 ± 0.05	1.64 ± 0.01	0.60 ± 0.18
16:1n-7	0.23 ± 0.01	9.40 ± 0.05	5.58 ± 0.11	0.12 ± 0.01	0.53 ± 0.00	n.d.
18:0	1.78 ± 0.04	2.09 ± 0.01	0.63 ± 0.01	1.59 ± 0.01	0.86 ± 0.00	0.54 ± 0.15
18:1n-9	61.94 ± 0.05	14.99 ± 0.03	5.91 ± 0.03	6.33 ± 0.02	2.44 ± 0.01	n.d.
18:1n-7	3.14 ± 0.03	4.78 ± 0.01	0.27 ± 0.05	0.12 ± 0.01	0.40 ± 0.00	n.d.
18:2n-6	18.40 ± 0.04	2.22 ± 0.01	tr.	2.32 ± 0.01	0.27 ± 0.00	n.d.
18:3n-3	7.07 ± 0.02	0.64 ± 0.01	tr.	tr.	0.12 ± 0.01	n.d.
20:1n-9	1.29 ± 0.03	14.52 ± 0.06	n.d.	tr.	2.87 ± 0.01	n.d.
20:5n-3	n.d.	9.86 ± 0.03	1.49 ± 0.01	15.69 ± 0.02	22.54 ± 0.07	n.d.
22:1n-9	0.24 ± 0.01	7.05 ± 0.29	n.d.	n.d.	1.92 ± 0.14	n.d.
22:5n-6	n.d.	n.d.	7.73 ± 0.02	5.75 ± 0.01	n.d.	n.d.
22:5n-3	n.d.	1.23 ± 0.06	0.21 ± 0.02	5.12 ± 0.01	5.33 ± 0.00	n.d.
22:6n-3	n.d.	13.29 ± 0.05	45.72 ± 0.12	39.11 ± 0.03	51.45 ± 0.06	98.86 ± 0.33
ΣSAFA	7.34 ± 0.07	16.49 ± 0.05	31.92 ± 0.06	23.37 ± 0.04	5.05 ± 0.03	1.14 ± 0.33
ΣMUFA	66.84 ± 0.01	51.13 ± 0.15	11.77 ± 0.13	6.60 ± 0.02	8.91 ± 0.12	–
ΣPUFA	25.83 ± 0.08	32.38 ± 0.10	56.32 ± 0.08	70.03 ± 0.04	86.04 ± 0.10	98.86 ± 0.33
DHA + EPA	–	23.15 ± 0.08	47.21 ± 0.12	54.80 ± 0.05	73.99 ± 0.13	98.86 ± 0.33
C_{avg}	17.97	18.80	19.24	19.96	20.51	21.94
ECN	16.71	16.70	15.86	16.09	15.79	16.02
$C{=}C_{avg}$	1.26	2.11	3.38	3.87	4.72	5.93
n-3/n-6	0.38 ± 0.00	8.3 ± 0.01	5.91 ± 0.01	6.54 ± 0.01	18.73 ± 0.02	–

tr.: traces; n.d.: not detected.

should be noted that, despite the importance of peristalsis for lipid digestion, a recommendation for shear force is absent in INFOGEST 2.0 protocol.

Fig. 1 reports the relative areas of TAGs, DAGs, and MAGs signals in undigested oil and digestate extracts. Fish oil concentrate was the only oil showing 1,2-DAG and 1-MAG signals (6.7% and 2.5%, respectively) prior to digestion, probably due to the TAG restructuring. After digestion, rapeseed oil had the highest relative amount of digestions products (57.2%), while tridocosahexaenoin had the lowest (3.5%). Despite the noticeable difference in composition (Table 1), there was no significant difference ($p > 0.05$) in the relative amounts of 1,2-DAG and 2-MAG between fish oil and fish oil concentrate. On the other hand, digestion products in microalgae oil 2 were found in higher relative amounts compared to either of fish oils (41% of total ^{1}H area against 9%). Also, compared to fish oil and fish oil concentrate, microalgae oil 2 had higher ($p < 0.05$) percentage of 1,2-DAG (15.7% against 10.2% and 12.2%, respectively) and 2-MAG (19.9% against 8.7% and 8.4%, respectively). At the end of the digestion, rapeseed oil had the highest total MAG/1,2-DAG ratio (3.9), followed by microalgae oils and tridocosahexaenoin (around 1.6), and fish oil and fish oil concentrate (around 1.1). The 2-MAG/1,2-DAG ratio followed the same trend, except for fish oil concentrate, which had the lowest ratio (0.7). Conversely, rapeseed oil had the highest reduction of ^{1}H TAG signal followed by microalgae oil 2 and fish oil, microalgae oil 1, fish oil concentrate, and tridocosahexaenoin. Therefore, there was no exact correspondence between TAG reduction and MAG production. Our observations led to the investigation of the influence of fatty acid composition, on one hand, and location of 22:6n-3 on the TAG structure, on the other, on oil lipolysis.

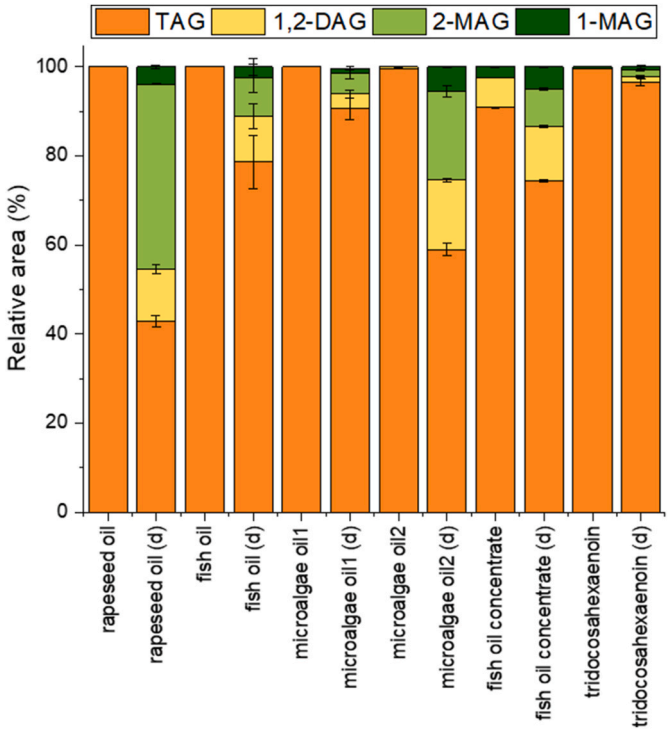

Fig. 1. Comparison of glycerides ^{1}H relative areas in the investigated oils and their digesta (marked with d). Oils are ordered in increasing PUFA (w/w %) content.

Fig. 2 reports the observed correlations between TAG ^{1}H area decrease and fatty acid composition of the oils. Fig. 2a highlighted the negative impact of n-3 fatty acids presence on the degree of hydrolysis of the oil. On the other hand, data reported in Fig. 2b, showing the relationship between TAG hydrolysis and n-6 and n-9 contents, hinted at a positive relationship between lipolysis and n-6 fatty acids content. Linear fitting of data points in Fig. 2 would be meaningless, as the degree of hydrolysis of rapeseed oil is the highest reachable at the present oil concentration. Also, the presence of a maximum degree of hydrolysis at a given oil concentration in simulated digestion can be derived from published data (Sabet et al., 2022; Tan et al., 2020). In addition, the non-linear trends reported in Fig. 2 were reasonably asymptotic to approximately the value of tridocosahexaenoin lipolysis, which was similar to fish oil supplement. The asymptotic trend in Fig. 2a hinted at the presence of a critical n-3/n-6 ratio for oil lipolysis (12.4), which can only be indicative as function fitting was only 0.600. Higher fittings were obtained with DHA + EPA (Fig. 2c), average carbon length (C_{avg}), and average amount of double bonds ($C{=}C_{avg}$, Fig. 2d). The respective critical points computed from the fitted trends were 44.5% w/w%, 19.2 average chain length, and 3.2 average unsaturation. These values were in partial agreement with each other.

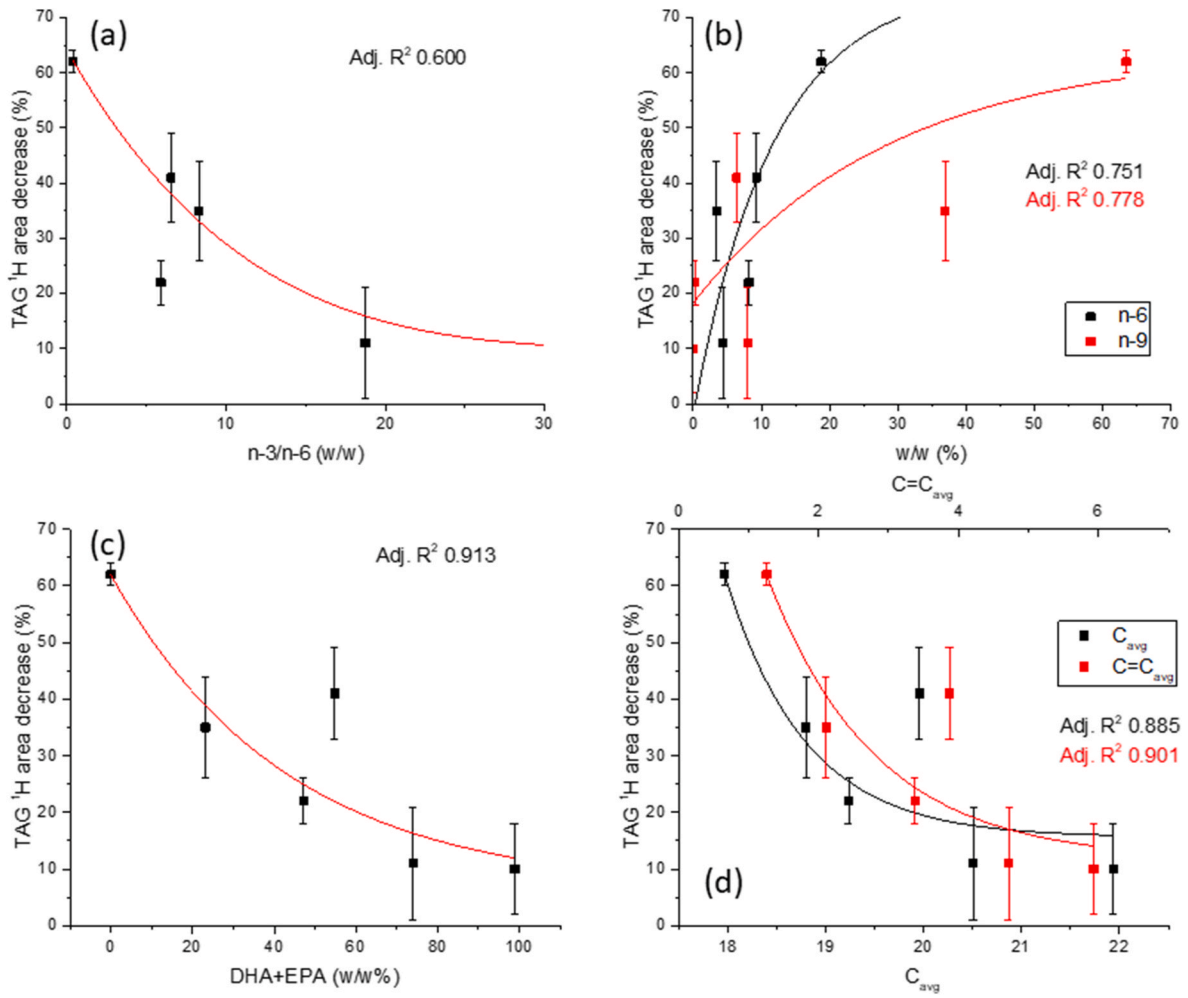

Fig. 2. Non-linear model of TAG hydrolysis, expressed as ^{1}H signal area decrease, in relationship with (a) weight ratio of total n-3/n-6 fatty acids; (b) relative amounts of n-6, n-9, and MUFA, expressed as relative weight %; (c) sum of relative amounts of 22:6n-3 (DHA) and 20:5n-3 (EPA); (d) average chain length of fatty acids (Cavg) and average number of double bonds (C=Cavg).

3.3. ^{13}C NMR: positional distribution of fatty acids and TAG hydrolysis

The positional distribution of fatty acids on the glycerol backbone of oil TAGs was analyzed with ^{13}C NMR, which can discern *sn*-2 from *sn*-1 and *sn*-3 positions. The present work has focused on the positioning of 22:6n-3 and 20:5n-3, following previously published instrumental parameters and signal assignments (Aursand et al., 2007). The integration results for positions *sn*-2 and *sn*-1,3 are reported in Fig. 3 in decreasing

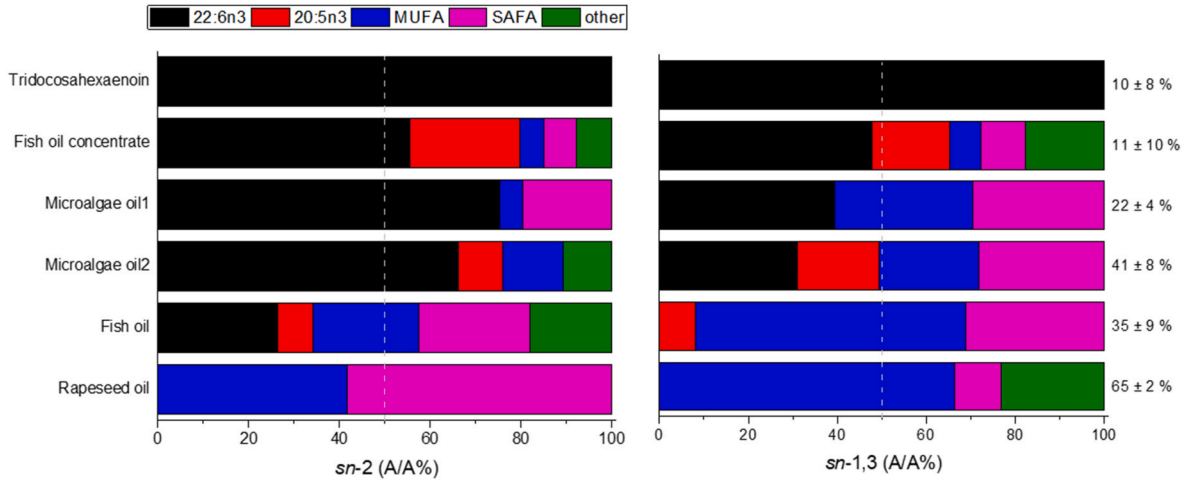

Fig. 3. Relative area percentage of fatty acids and their positional distribution in the investigated oils by ^{13}C NMR. Percentages adjacent to the bars represent TAG hydrolysis, expressed as ^{1}H signal area decrease. Oils are ordered in decreasing DHA *sn*-1,3 percentage.

order of 22:6n-3 in position *sn*-1,3. A good correspondence in 22:6n-3 and 20:5n-3 relative amounts between ^{13}C NMR and chromatographic data was observed (Supplementary Fig. 2). Noticeably, the halving of 22:6n-3 relative signal area in *sn*-1,3 from tridocosahexaenoin to fish oil concentrate had no observable improvement of TAG lipolysis, while improvement in lipolysis was observed when 22:6n-3 relative signal area decreased to 39%. This observation led to the computation of the non-linear relationship between TAG ^{1}H area decrease and 22:6n-3 relative signal area in positions *sn*-1,3, which are the ones hydrolyzed during digestion. The results, reported in Fig. 4 showed decent fitting (adjusted R^2 0.844). A clear critical amount of 22:6n-3 was absent. Fish oil represented an outlier in the trend as it lacked 22:6n-3 in positions *sn*-1,3. Possibly, the complexity of the system (i.e., oxidation status of the oil), coupled to an inadequate amount of data points, hid the presence of a critical amount, which would be found at 38% A/A% by removal of the outlier, close to the one deduced from GC-FID data (DHA + EPA 45% w/w, Supplementary Fig. 3). Understanding of the system is further complicated by the presence of 20:5n-3 in *sn*-1,3 positions of fish oil, fish oil concentrate, and microalgae oil 2 (Fig. 3), which has as well a negative impact on lipolysis (Beltrame et al., 2023).

3.4. Significance for in vitro and in vivo studies

There in an increasing interest in incorporation of n-3 LC-PUFAs in new food formulas, and the investigation on digestibility and absorption of these essential fatty acids has correspondingly increased in relevance. However, the present results have showed, regardless of the hypothetical critical 22:6n-3 amount in *sn*-1,3, a potentially inadequate lipolysis with INFOGEST 2.0 model of oils having natural or synthetic prevalence of 22:6n-3 in *sn*-1,3 positions, at least at concentrations useful for detailed chemical analysis of digestion products. Relevant oils with 22:6n-3 largely positioned in *sn*-1,3 originate from thraustochytrids (Lee Chang et al., 2014), harp seal, tuna (Tengku-Rozaina & Birch, 2014), or TAG restructuring (Damerau et al., 2020).

The release of fatty acids from TAGs is one of the contributors to lipid oxidation in the gastrointestinal tract. Therefore, lipolysis should be taken into account when lipid oxidation at simulated digestion conditions is examined. Our previous work showed that hydrolysis of PUFA-containing TAGs to DAGs increased PUFA hydroperoxidation, i.e. had deprotective effect (Beltrame et al., 2023). At the same time, free fatty acids are more labile to oxidation compared to esterified forms (Lyberg et al., 2005), although their contribution to oxidation damage in the simulated intestine is unclear (Tullberg & Undeland, 2021). Tullberg et al. showed that increase in lipolysis led to an increase in secondary oxidation products during simulated digestion of marine oils (Tullberg et al., 2019). Therefore, understanding of oxidation behavior of n-3-rich oils in the gastrointestinal tract requires realistic lipolysis.

Present results raise questions also regarding *in vivo* studies, as most of them lack attention to the location of fatty acids in the TAG backbone. Human trials with *Schizochytrium* sp oil focused solely on blood DHA concentration (García-Maldonado et al., 2023) but results from an animal trial published by our group showed poorer absorption of DHA located in *sn*-1 and *sn*-3 positions compared to *sn*-2 (Linderborg et al., 2019). The results of this short communication call for further exploration of the physiological relevance of these findings by controlled animal and human studies.

4. Conclusions

Simulated digestion of n-3 LC-PUFA-rich oils according to INFOGEST 2.0 showed considerably lower degree of lipolysis compared to rapeseed oil. Collected data of rapeseed and n-3 LC-PUFA-rich oils showed negative non-linear relationships between lipolysis and oil n-3/n-6 fatty acid ratio and relative amount of 22:6n-3 in TAG *sn*-1,3 position. Such obstacle should be taken into account during *in vitro* research on digestibility and absorption of long chain n-3-rich oils.

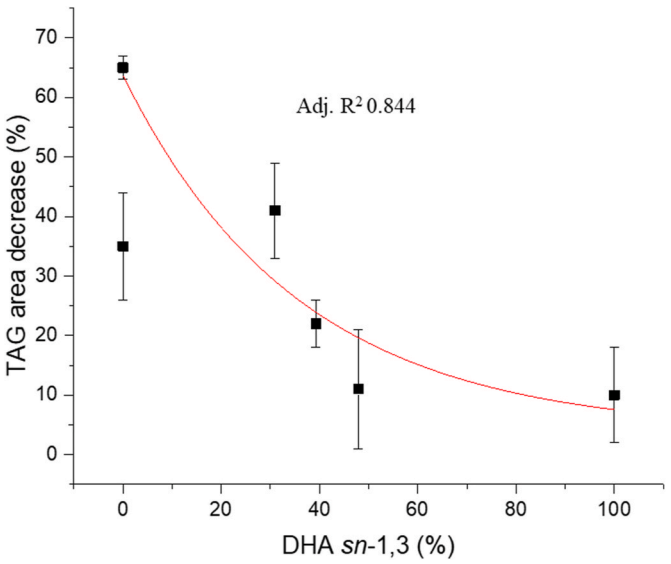

Fig. 4. Non-linear model of TAG hydrolysis, expressed as ^{1}H signal area decrease, and relative amount of DHA in TAG *sn*-1,3 positions expressed as ^{13}C relative signal area.

CRediT authorship contribution statement

Gabriele Beltrame: Writing – original draft, Visualization, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Iida Valta:** Writing – review & editing, Investigation. **Annelie Damerau:** Writing – review & editing, Investigation. **Kaisa M. Linderborg:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Funding

This work was carried out as part of the project "Omics of oxidation – Solutions for better quality of docosahexaenoic and eicosapentaenoic acids" funded by the Academy of Finland (grant number 315274, PI Kaisa Linderborg). Gabriele Beltrame acknowledges personal financial grants from Niemi Foundation and from Finnish Cultural Foundation.

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.

Acknowledgements

The manuscript benefited from discussions with Prof. Ingrid Undeland and Dr. Karin Larsson from Food and Nutrition Science division of Chalmers University of Technology (Gothenburg, Sweden). The authors thank Hafdís Haraldsdóttir for her help in the interpretation of the NMR spectra and Klara Helgesson for her help in the measurement of the lipase activities.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2025.117835.

Data availability

Data will be made available on request.

References

Aursand, M., Standal, I. B., & Axelson, D. E. (2007). High-resolution 13C nuclear magnetic resonance spectroscopy pattern recognition of fish oil capsules. *Journal of Agricultural and Food Chemistry, 55*(1), 38–47. https://doi.org/10.1021/jf061754l

Beltrame, G., Ahonen, E., Damerau, A., Gudmundsson, H. G., Haraldsson, G. G., & Linderborg, K. M. (2023). Lipid structure influences the digestion and oxidation behavior of docosahexaenoic and eicosapentaenoic acids in the simulated digestion system. *Journal of Agricultural and Food Chemistry, 71*(26), 10087–10096. https://doi.org/10.1021/acs.jafc.3c02207

Beltrame, G., Damerau, A., Ahonen, E., Mustonen, S. A., Adami, R., Sellitto, M. R., Del Gaudio, P., & Linderborg, K. M. (2024). Production and simulated digestion of high-load beads containing *Schizochytrium* oil encapsulated utilizing prilling technique. *Food Chemistry, 460*(P3), Article 140694. https://doi.org/10.1016/j.foodchem.2024.140694

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., … Recio, I. (2019). INFOGEST static *in vitro* simulation of gastrointestinal food digestion. *Nature Protocols, 14*(4), 991–1014. https://doi.org/10.1038/s41596-018-0119-1

Damerau, A., Ahonen, E., Kortesniemi, M., Puganen, A., Tarvainen, M., & Linderborg, K. M. (2020). Evaluation of the composition and oxidative status of omega-3 fatty acid supplements on the Finnish market using NMR and SPME-GC–MS in comparison with conventional methods. *Food Chemistry, 330*(June), Article 127194. https://doi.org/10.1016/j.foodchem.2020.127194

García-Maldonado, E., Alcorta, A., Zapatera, B., & Vaquero, M. P. (2023). Changes in fatty acid levels after consumption of a novel docosahexaenoic supplement from algae: A crossover randomized controlled trial in omnivorous, lacto-ovo vegetarians and vegans. *European Journal of Nutrition, 62*(4), 1691–1705. https://doi.org/10.1007/S00394-022-03050-3/FIGURES/3

Gayoso, L., Ansorena, D., & Astiasarán, I. (2019). DHA rich algae oil delivered by O/W or gelled emulsions: Strategies to increase its bioaccessibility. *Journal of the Science of Food and Agriculture, 99*(5), 2251–2258. https://doi.org/10.1002/jsfa.9420

Gomes, R., Martins, S., Afonso, C., Bandarra, N. M., & Cardoso, C. (2019). Comparison of fish and oil supplements for a better understanding of the role of fat level and other food constituents in determining bioaccessibility. *Food Science and Nutrition, 7*(4), 1179–1189. https://doi.org/10.1002/fsn3.894

Grundy, M. M. L., Abrahamse, E., Almgren, A., Alminger, M., Andres, A., Ariëns, R. M. C., Bastiaan-Net, S., Bourlieu-Lacanal, C., Brodkorb, A., Bronze, M. R., Comi, I., Couëdelo, L., Dupont, D., Durand, A., El, S. N., Grauwet, T., Heerup, C., Heredia, A., Infantes Garcia, M. R., … Carrière, F. (2021). INFOGEST inter-laboratory recommendations for assaying gastric and pancreatic lipases activities prior to in vitro digestion studies. *Journal of Functional Foods, 82*(May), Article 104497. https://doi.org/10.1016/j.jff.2021.104497

Halldorsson, A., Magnusson, C. D., & Haraldsson, G. G. (2003). Chemoenzymatic synthesis of structured triacylglycerols by highly regioselective acylation. *Tetrahedron, 59*(46), 9101–9109. https://doi.org/10.1016/J.TET.2003.09.059

Lee Chang, K. J., Nichols, C. M., Blackburn, S. I., Dunstan, G. A., Koutoulis, A., & Nichols, P. D. (2014). Comparison of thraustochytrids aurantiochytrium sp., Schizochytrium sp., Thraustochytrium sp., and Ulkenia sp. for production of biodiesel, long-chain omega-3 oils, and exopolysaccharide. *Marine Biotechnology, 16*(4), 396–411. https://doi.org/10.1007/S10126-014-9560-5/FIGURES/6

Linderborg, K. M., Kulkarni, A., Zhao, A., Zhang, J., Kallio, H., Magnusson, J. D., Haraldsson, G. G., Zhang, Y., & Yang, B. (2019). Bioavailability of docosahexaenoic acid 22:6(n-3) from enantiopure triacylglycerols and their regioisomeric counterpart in rats. *Food Chemistry, 283*, 381–389. https://doi.org/10.1016/j.foodchem.2018.12.130

Lyberg, A., Fasoli, E., & Adlercreutz, P. (2005). Monitoring the oxidation of docosahexaenoic acid in lipids. *Lipids, 40*(9). https://doi.org/10.1007/s11745-005-1458-1

Nieva-Echevarría, B., Goicoechea, E., Manzanos, M. J., & Guillén, M. D. (2016). A study by 1H NMR on the influence of some factors affecting lipid *in vitro* digestion. *Food Chemistry, 211*, 17–26. https://doi.org/10.1016/j.foodchem.2016.05.021

RStudio. (2020). *RStudio: Integrated development for R*. Boston, MA: RStudio, Inc.. *URL* http://www.rstudio.com/.

Sabet, S., Kirjoranta, S. J., Lampi, A.-M., Lehtonen, M., Pulkkinen, E., & Valoppi, F. (2022). Addressing criticalities in the INFOGEST static *in vitro* digestion protocol for oleogel analysis. *Food Research International, 160*, Article 111633. https://doi.org/10.1016/J.FOODRES.2022.111633

Saini, R. K., & Keum, Y. S. (2018). Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance — a review. *Life Sciences, 203*(January), 255–267. https://doi.org/10.1016/j.lfs.2018.04.049

Tan, Y., Zhang, Z., Zhou, H., Xiao, H., & McClements, D. J. (2020). Factors impacting lipid digestion and β-carotene bioaccessibility assessed by standardized gastrointestinal model (INFOGEST): Oil droplet concentration. *Food & Function, 11*(8), 7126–7137. https://doi.org/10.1039/D0FO01506G

Tan, Y., Zhou, H., & McClements, D. J. (2022). Application of static *in vitro* digestion models for assessing the bioaccessibility of hydrophobic bioactives: A review. *Trends in Food Science & Technology, 122*, 314–327. https://doi.org/10.1016/j.tifs.2022.02.028

Tengku-Rozaina, T. M., & Birch, E. J. (2014). Positional distribution of fatty acids on hoki and tuna oil triglycerides by pancreatic lipase and 13 C NMR analysis. *European Journal of Lipid Science and Technology, 116*(3), 272–281. https://doi.org/10.1002/ejlt.201300357

Tullberg, C., & Undeland, I. (2021). Oxidative stability during digestion. In *Omega-3 delivery systems* (pp. 449–479). Elsevier. https://doi.org/10.1016/B978-0-12-821391-9.00008-9.

Tullberg, C., Vegarud, G., & Undeland, I. (2019). Oxidation of marine oils during *in vitro* gastrointestinal digestion with human digestive fluids – role of oil origin, added tocopherols and lipolytic activity. *Food Chemistry, 270*(May 2018), 527–537. https://doi.org/10.1016/j.foodchem.2018.07.049

Versantvoort, C. H. M., Oomen, A. G., Van de Kamp, E., Rompelberg, C. J. M., & Sips, A. J. A. M. (2005). Applicability of an in vitro digestion model in assessing the bioaccessibility of mycotoxins from food. *Food and Chemical Toxicology, 43*(1), 31–40. https://doi.org/10.1016/j.fct.2004.08.007