Behaviour of n-3 polyunsaturated fatty acid ethyl esters in INFOGEST *in vitro* model

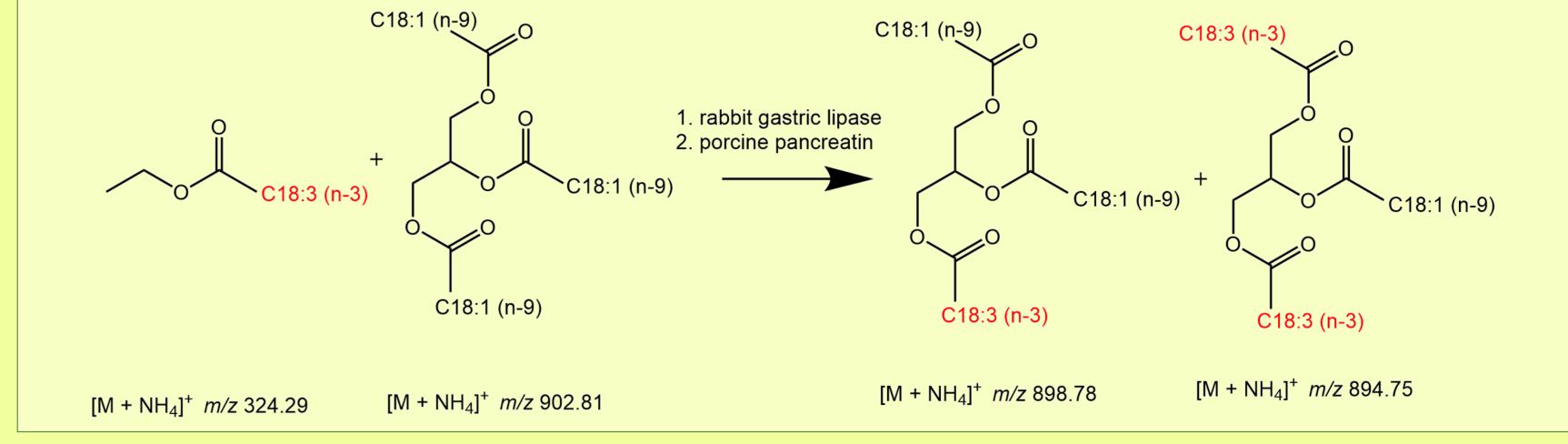
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Introduction and aim

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Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are crucial for optimal neural development and for human health. They can reduce inflammation and prevent cardiovascular diseases. Plant and seed oils are rich sources of α -linolenic acid (ALA, C18:3n-3). Oxidation decreases the bioavailability and sensory properties of n-3 PUFAs. Highly oxidation prone n-3 oils can be protected by microencapsulation. Content of n-3 PUFAs in supplements is often enriched in ethyl ester (EE) form by transesterification reaction. Shortterm clinical studies have shown reduced bioavailability of n-3 PUFA EE compared to natural triacylglycerol (TAG) form, possibly due to restricted hydrolysis by lipase in gastrointestinal tract. Ingestion of EEs with high-fat meal is known to improve their bioavailability.

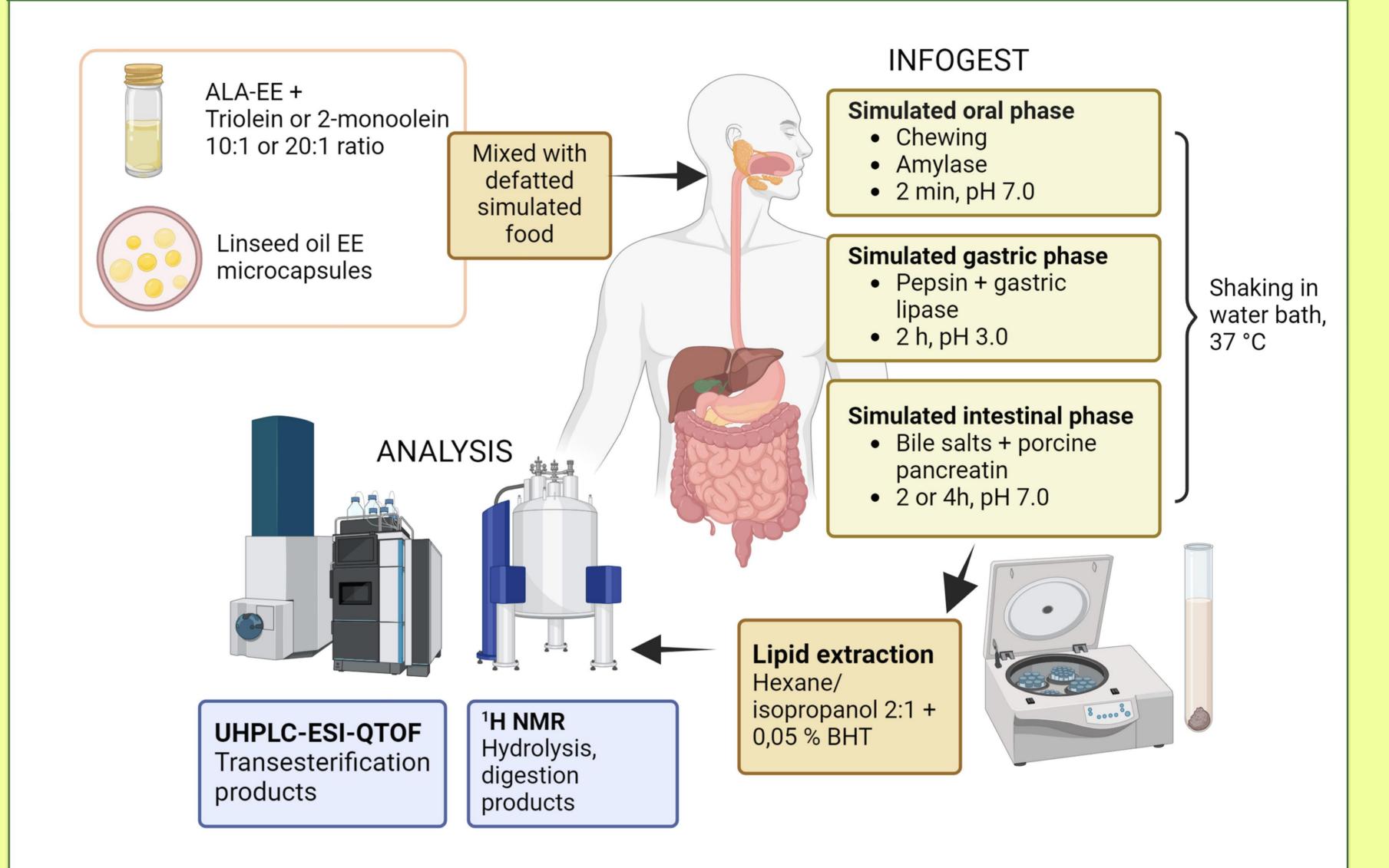


Aim was to study digestion of ALA-EE in the presence of dietary fat with INFOGEST *in vitro* digestion model. It was investigated whether EEs are transesterified with dietary acylglycerols in simulated intestinal lumen (**Fig 1**). Since influence of polysaccharide-type wall materials on bioavailability is not well understood, also microencapsulated linseed oil EEs were digested.



Figure 1. Predicted transesterification reaction

Materials and methods



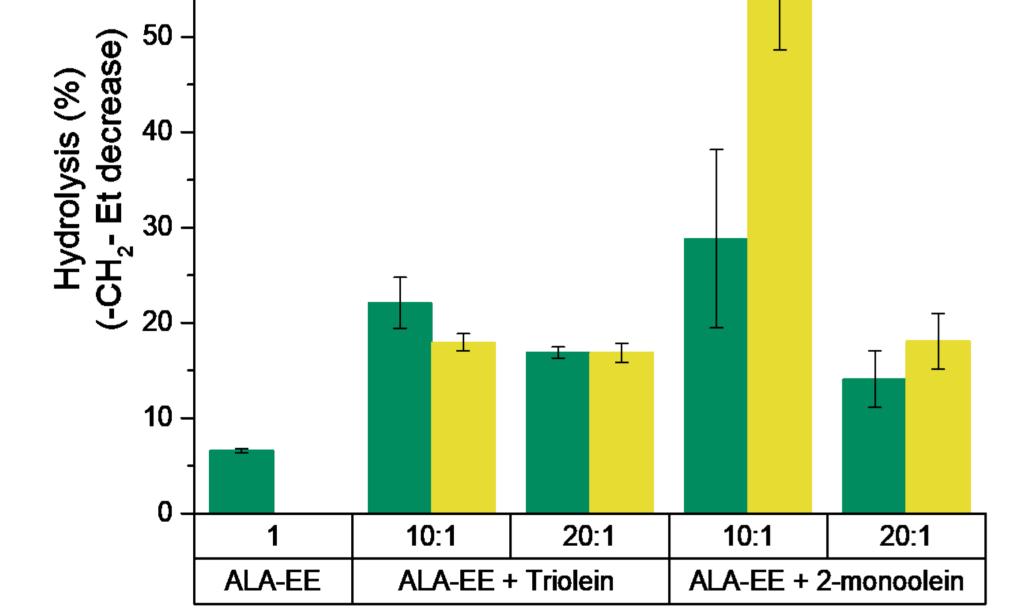


Figure 3. Hydrolysis of digested samples (n=3) analyzed with ¹H NMR

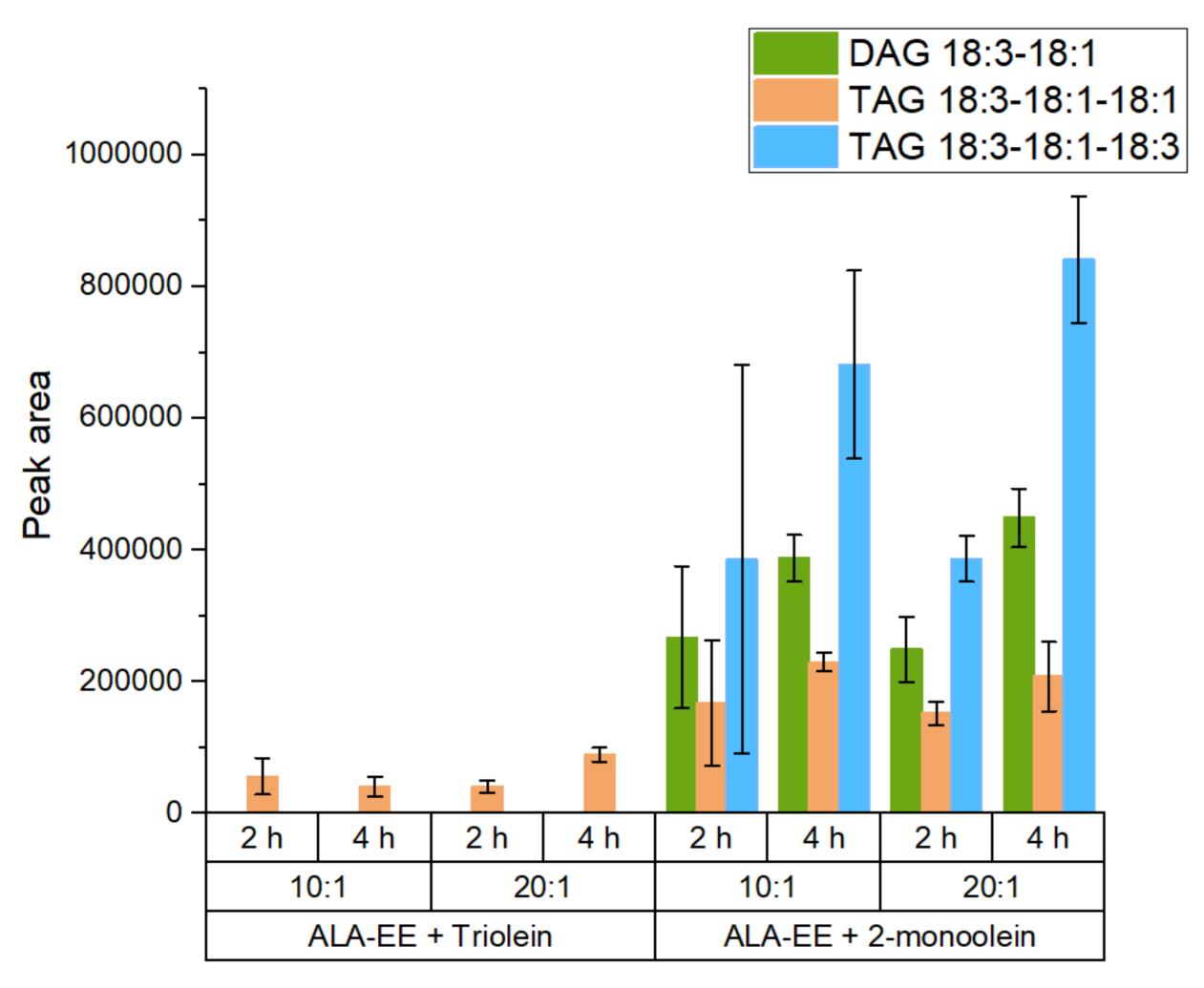


Figure 2. Schematic diagram of materials and methods. Linseed oil EE microcapsules produced by spray drying in University of Warmia and Mazury (Poland), coating materials: maltodextrin (M), native (N) or gelatinized (G) potato (P) or rice starches (R)

Table 1. Ratio of sum of transesterifiedproducts and 2-monoolein

| Digestion parameters | Transesterified products / 2-monoolein (peak areas) | |
|-------------------------|--|--------------------|
| | UHPLC-ESI- QTOF | ¹ H NMR |
| 2-monoolein 10:1 2h | 0.90 ± 0.64 | 0.39 ± 0.23 |
| 2-monoolein 10:1 4h | 2.14 ± 0.28 | 1.06 ± 0.10 |
| 2-monoolein 20:1 2h | 0.99 ± 0.17 | 0.45 ± 0.12 |
| 2-monoolein 20:1 4h | 2.28 ± 0.51 | 0.73 ± 0.08 |

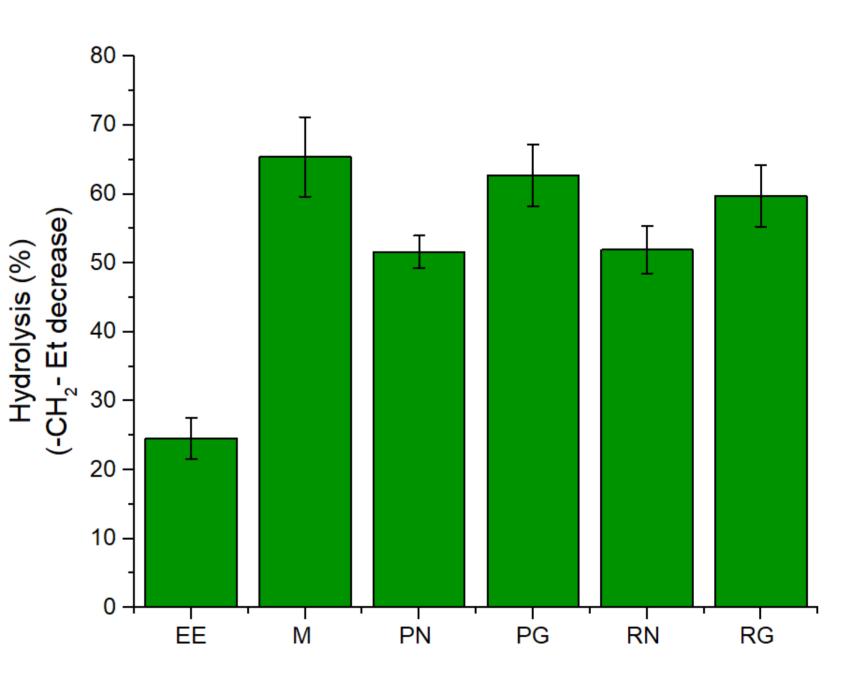


Figure 5. Hydrolysis of native and microencapsulated linseed oil EEs (n=3, 2 h intestinal phase)

Figure 4. Peak areas of transesterified products in digested samples (n=3) analyzed with UHPLC-ESI-QTOF

Conclusions

- Hydrolysis and transesterification of ALA-EE was highest in the presence of 2monoolein and with prolonged intestinal phase.
- Transesterification seems not to be significant route in absorption of EEs.
- Most likely improved hydrolysis in the presence of 2-monoolein or microencapsulation occurs due to higher emulsification, which is essential for lipase activity.
- Starches are suitable coating materials in terms of digestibility.
- More precise picture of absorption could be obtained by introducing Caco2cells which model intestinal epithelial barrier.

References

Schuchardt JP and Hahn A (2013) *Prostaglandins Leukot Essent Fatty Acids*, 89(1):1-8. doi: 10.1016/j.plefa.2013.03.010 Ogrodowska D et al (2024) *Journal of Food Engineering* 364:111799 doi: 10.1016/j.jfoodeng.2023.111799 Brodkorb A et al (2019) Nat Protocols 14(4):991-1014, DOI:10.1038/s41596-018-0119-1 Castejón N and Señoráns FJ (2019). *European Journal of Lipid Science and Technology* 121(5):1800412. doi: 10.1002/ejlt.201800412