Lipid oxidation during instrumented dynamic in vitro digestion of marine oil-enriched milk

Meynier, A.; Jacobsen, Charlotte; Thomsen, Birgitte Raagaard; Brestaz, P.; Ribourg, L.; Viau, M.; Genot, C.

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Lipid oxidation during instrumented dynamic in vitro digestion of marine oil-enriched milk

A. Meynier, C. Jacobsen♣, B.R. Thomsen♣, P. Brestaz, L. Ribourg, M. Viau, C. Genot,

The health benefits of n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) are well recognized. Foods enriched in n-3 LC-PUFA, are now available for the consumers. However, PUFA are prone to oxidation during processing and storage of the enriched-foods, but also during their digestion, and deleterious compounds can be formed both during both gastric and intestinal steps [1].

The aim of this study was to evaluate the formation of toxic aldehydes: malondialdehyde (MDA), 4-hydroxy-2-hexenal (4-HHE), 4-hydroxy-2-nonenal (4-HNE) during the *in vitro* digestion of marine oil enriched milk.

Material & Methods

Milk enrichment

Fish oil 0.5 % (w/w)
PV = 2.4 meq/kg ; AV = 7.55
MDA: 68 nmole/g oil
4-HHE: 0.8 nmole/g oil oil
4-HNE: 0.18 nmole/g oil
Tocopherols : 890 µg/g oil
EPA + DHA : 11 % total fatty acids
n-6/n-3 PUFAs : 0.26

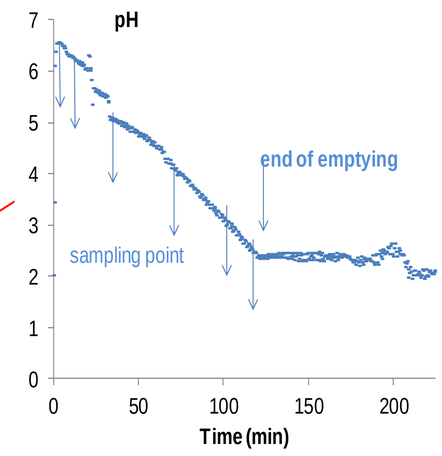
Homogenized pasteurized **milk**
1% fat
→ Homogenization (300 bars, 2.5 min)

Marine-oil enriched milk
1% milk fat, 0.5% fish oil

DiDGI®

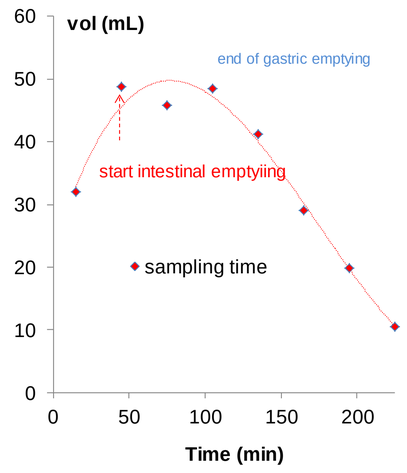
Ingestion rate (100mL/3 min)

Stomach



Intestine

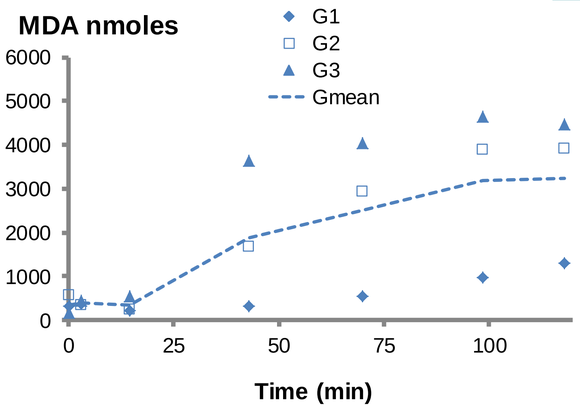
pH regulated at 6.5



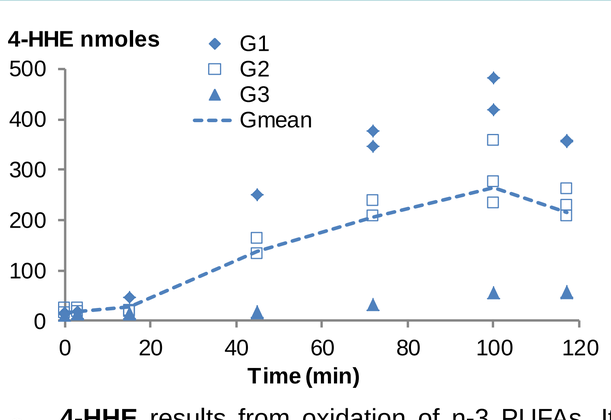
Results & Discussion

Three digestions were performed independently on three marine oil-enriched milks prepared separately.
MDA, 4-HHE and 4-HNE were measured on the samples from the 3 experiments. The individual data obtained for MDA (left), 4-HHE (middle) and 4-HNE (right) during the gastric (blue symbols, G1, G2, G3) and intestinal steps (red symbols, I1, I2, I3) of each digestion are shown. The blue and red lines locate the mean values.
Total amounts in each compartment (nmoles) calculated from volumes of digestive media are presented.

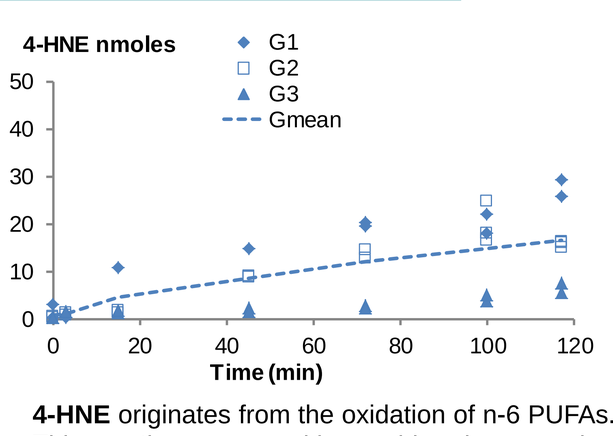
Lipid oxidation in the stomach compartment



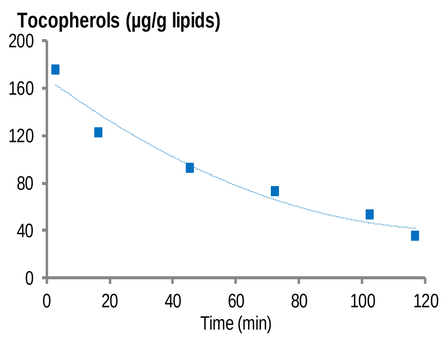
MDA can result from the oxidation of both n-6 and n-3 PUFAs. It is toxic for cells (2,3). It was produced in fairly high concentrations (75 µM at the end of the gastric phase) which corresponds to around 4 µmoles in the total digestive fluid and 1.2 µmoles/g ingested lipids



4-HHE results from oxidation of n-3 PUFAs. It is very reactive and binds to proteins. It can be absorbed by the intestinal cells and provokes oxidative stress (4). It was produced continuously in the acid gastric environment. It represented up to 7 µM digestive medium, meaning total of 400 nmoles and around 800 nmoles/g lipids.



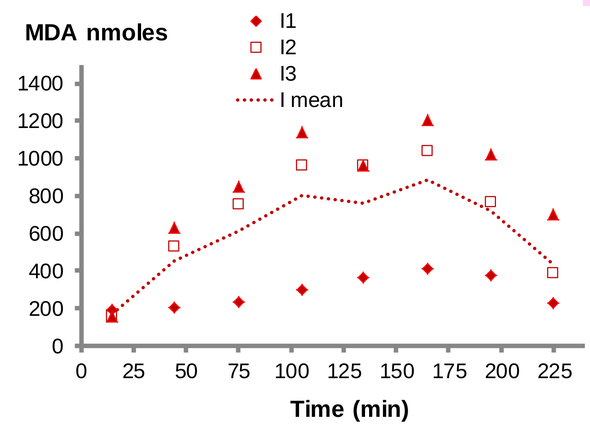
4-HNE originates from the oxidation of n-6 PUFAs. This reactive compound is considered as a marker of oxidative stress *in vivo* (2). In accordance with the fatty acid composition of the fish oil, poor in n-6 PUFAs, its amounts remained fairly low as compared to MDA and 4-HHE: around 0.3 µM, 20 nmoles and 50 nmoles/g lipids.

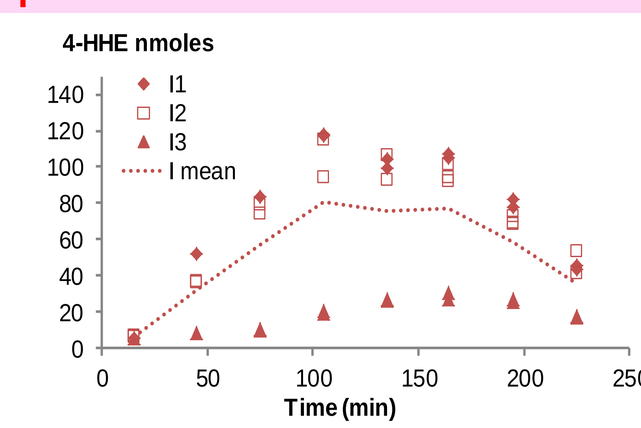


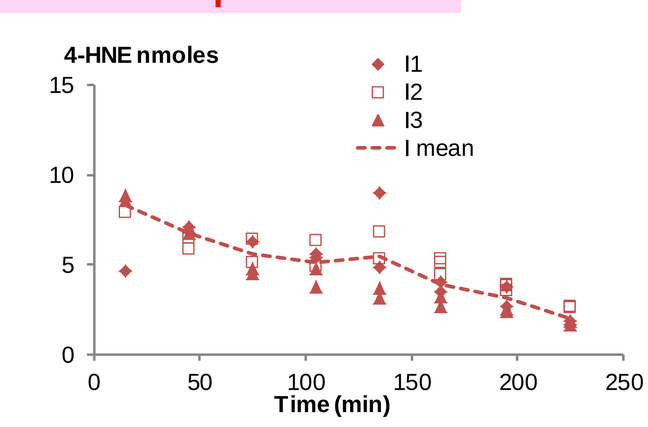
During the gastric step, toxic aldehydes such as MDA and 4-HHE were formed while tocopherols were consumed.

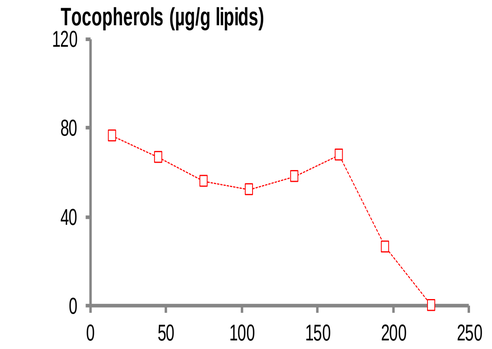
The results confirm that oxidation of the n-3 PUFAs of marine oil-enriched milk took place in conditions close to real gastric conditions (pH variation, 5), even in the absence of oxidation initiator such as heme iron.

Lipid oxidation in the intestine compartment









MDA and **4-HHE** concentrations increased continuously in the intestinal conditions. However, their concentrations were lower than in the gastric medium. It could result from the dilution by the digestive fluids and from the binding of the reactive aldehydes to the components of the medium, including the hydrolyzed milk proteins. The decrease of MDA and 4-HHE total amounts at the end of the intestinal step is linked to the decrease of the fluid volume (emptying). **4-HNE** was not appreciably produced during intestinal digestion of marine oil-enriched milk.

Oxidation and production of MDA and 4-HHE continued during the intestinal simulated digestion.

Tocopherol concentrations remained fairly stable during most of the intestinal step. The decrease observed after 165 min is tentatively attributed to the high dilution of the lipids contained in the meal in those belonging to the digestive fluids .

Conclusions

Toxic aldehydes (MDA >> 4-HHE >> 4-HNE) were formed during the gastric and intestinal steps of the *in vitro* digestion of the marine oil-enriched milk. Tocopherols were consumed mostly during the gastric step. Results must be confirmed by in vivo studies, to evaluate in the case of n-3 LC-PUFA enriched-foods, the health effects of the oxidative reactions during digestion.

References

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