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DIFFERENCES BETWEEN THE OXIDATION PROCESSES OF TWO DIETARY SUPPLEMENTS RICH IN OMEGA-3 LIPIDS WITH SIMILAR UNSATURATION DEGREE. ROLE OF MINOR COMPONENTS.

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INTRODUCTION

A wide variety of dietary supplements rich in highly polyunsaturated long-chain omega-3 (ω-3) lipids like EPA and DHA are available on the market. They differ not only in their composition in main components (acyl groups), but also in that of minor ones, which usually include compounds added as antioxidants. Although these differences between supplements may influence their oxidative stability (1), little is known about their behaviour under oxidative conditions and the factors that can influence it.

OBJECTIVE

To **study** and **compare** the **behaviour** under mild **oxidative conditions** of **two non-encapsulated supplements** with very **similar** proportion of ω-3 groups and **unsaturation degree**, but **differing in certain minor components**, in order to **assess** to what **extent these latter** can **influence** their **oxidation process**.

METHODOLOGY

- **Samples**: Two dietary non-encapsulated supplements rich in ω-3 lipids manufactured from fish oil, with very similar contents of ω-3 groups: **S1**, richer in EPA and with a tocopherol mixture added, and **S2**, richer in DHA and with α-tocopherol.
- **Oxidation:** Samples (4 g, placed in 12 ml-vials) were subjected to mild accelerated storage conditions at 45º C in an oven with aeration, and aliquots were taken periodically throughout 114 days.
- **Study of the supplements and of their oxidation process:**
  - **^{1}H Nuclear Magnetic Resonance (^{1}H NMR)** was used to study supplement acyl group composition and unsaturation degree (ratio between saturated and unsaturated protons, $R_{S/U}$), as well as to follow their oxidation process through the evolution of EPA and DHA groups and the generation of certain oxidation products.
  - **Direct Immersion Solid Phase Microextraction (DI-SPME)** followed by **Gas Chromatography/Mass Spectrometry (GC/MS)** was used to get information about potentially antioxidant tocopherols present in the samples.

RESULTS AND DISCUSSION

1. SUPPLEMENT CHARACTERIZATION

Acyl groups: According to ^{1}H NMR data, **S1** and **S2** supplements contain ≈33% of ω-3 groups (molar percentage), EPA proportion being higher in **S1** and that of DHA more elevated in **S2** (see **Table 1**), in line with label information. The molar proportion of total unsaturated acyl groups was similar in both supplements, and despite their different EPA and DHA relative proportions, their unsaturation degrees ($R_{S/U}$) were very similar.

Table 1. Mean values of molar percentages of total ω-3 acyl groups, EPA, DHA and total unsaturated (TU) ones, unsaturation degree ($R_{S/U}$), estimated by ^{1}H NMR, and average abundances of the main tocopherols (area counts x 10^{-6} of the base peak of the mass spectrum), determined by DI-SPME-GC/MS.

	% ω-3	% EPA	% DHA	% TU	$R_{S/U}$	γ-T	α-T	δ-T	α-T acetate
S1	32.6	18.2	10.0	69.2	5.6	47.5	23.4	16.5	-
S2	33.2	9.7	24.4	68.3	5.5	87.3	25.4	38.7	94.1

Minor components: Although α-T was the only tocopherol stated on the label of **S2** sample, γ-T and δ-T were also present, together with α-T acetate, which showed the highest abundance and was absent from **S1**. Moreover, the levels of γ-T and δ-T were noticeably higher in **S2** than in **S1** (see **Table 1**).

2. SUPPLEMENT OXIDATION PROCESS

2.1. Evolution of EPA and DHA acyl groups: The two supplements showed different evolutions throughout the accelerated storage process (see **Figure 1**). Although in both cases a decrease of EPA and DHA group concentrations occurs with time, in **S1 sample** two distinct stages can be observed (see in **Figure 1** the values of the slopes of the equations to which EPA and DHA group evolutions are fitted): a first stage until day 49 and a second one from here onwards, in which the degradation rate of EPA and DHA groups is accelerated considerably, especially that of the former. In contrast, in **S2 sample**, the concentration decreasing rates of both DHA and EPA can be considered almost constant over time and lower than in **S1**, except in the case of DHA groups during the first 50 days, which decrease more rapidly in **S2** (see **Figure 1**). Consequently, the degradation extent of EPA and DHA groups after 60 days and up to the end of the studied period was higher in **S1** sample.

2.2. Oxidation product generation: Among the oxidation derivatives formed, compounds supporting hydroperoxy groups and exhibiting conjugated (*Z*,*E*)-dienes (HPO-c(*Z*,*E*)-dEs) were the first detected. The evolution of their concentration during the initial 50 days of the accelerated storage process is depicted in **Figure 2**. HPO-c(*Z*,*E*)-dEs are generated faster and reach a higher level in **S2** sample than in **S1**. This could be explained by the higher degradation rate of DHA groups in **S2** during this period (see **Figure 1**).

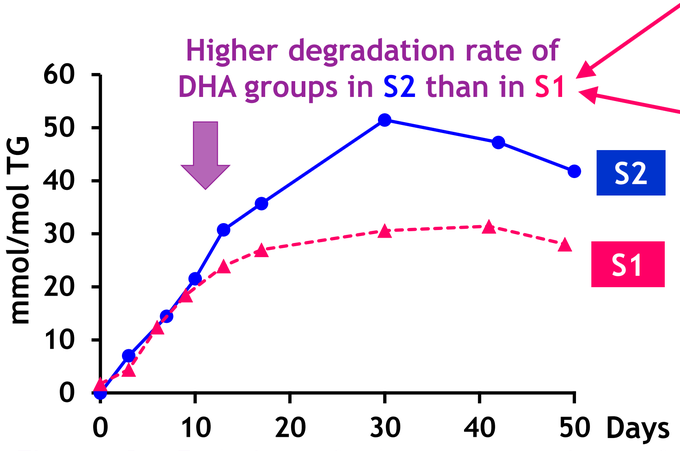

Figure 2. Evolution of the concentrations of hydroperoxy conjugated-(*Z*,*E*)-dienes during 50 days under oxidative conditions, expressed in millimoles per mole of triglyceride (mmol/mol TG).

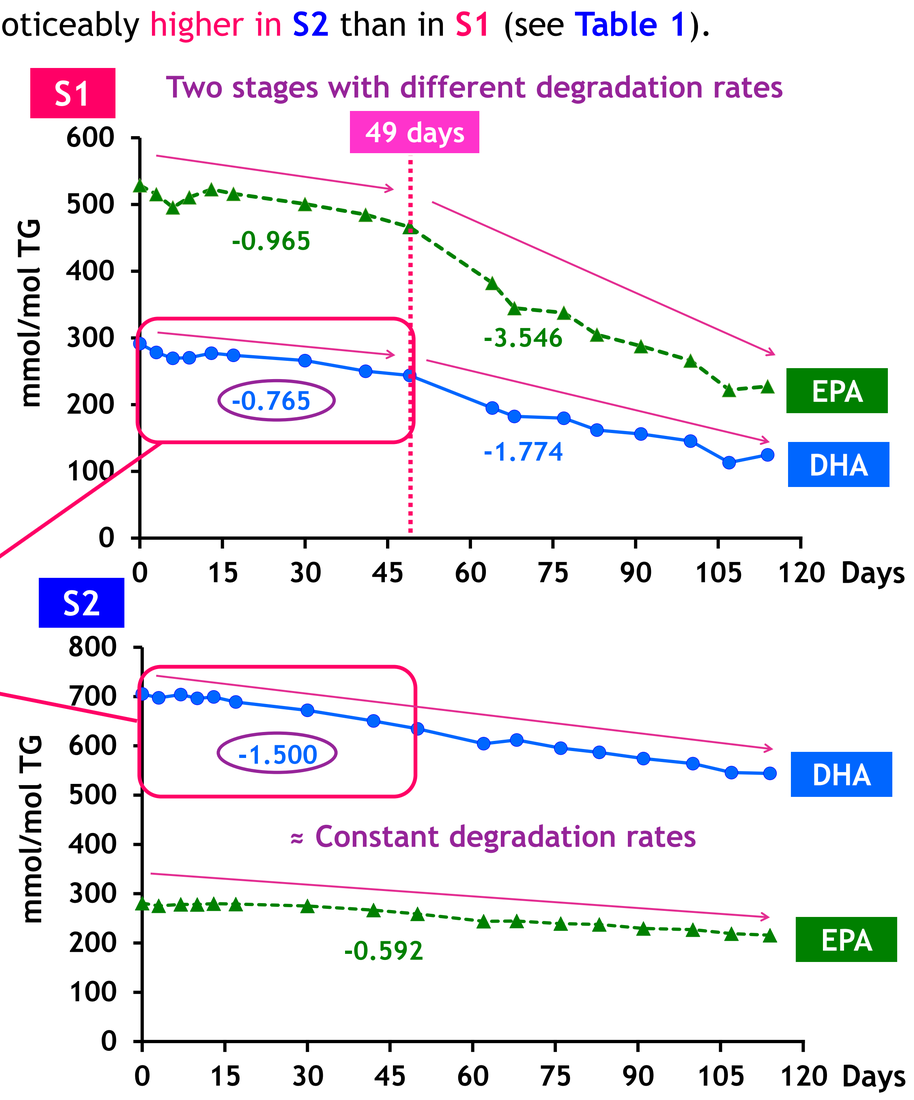

Figure 1. Evolution of the concentrations of EPA and DHA acyl groups, expressed in millimoles per mole of triglyceride (mmol/mol TG), throughout 114 days under accelerated storage conditions.

CONCLUSIONS

- **Dietary supplements** with similar contents of highly polyunsaturated long-chain ω-3 lipids and unsaturation degrees can show considerably **different evolutions** under mild oxidative conditions **depending on** their **composition** in **minor potentially antioxidant components**.
- Although during a first period the degradation rate of DHA groups and the generation of hydroperoxides are higher in **S2** than in **S1**, at the end of the oxidation process the loss of ω-3 groups is lower in the former. This means that **oxidative stability studies** of this type of supplements should be **carefully planned**, since **varying conclusions** could be drawn **depending on** their **duration**.
- These findings show the **great importance** of the **selection** of the **minor components added** to this kind of supplements in order to preserve their ω-3 lipid content, and the **need** for **further studies** to delve into the effect of the added compounds on ω-3 supplement stability.

REFERENCE

1. Abuzaytoun R, Shahidi F (2006) *J Agric Food Chem*, 54, 8253-8260.

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