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María Roca
Tenured scientist
Instituto de la Grasa

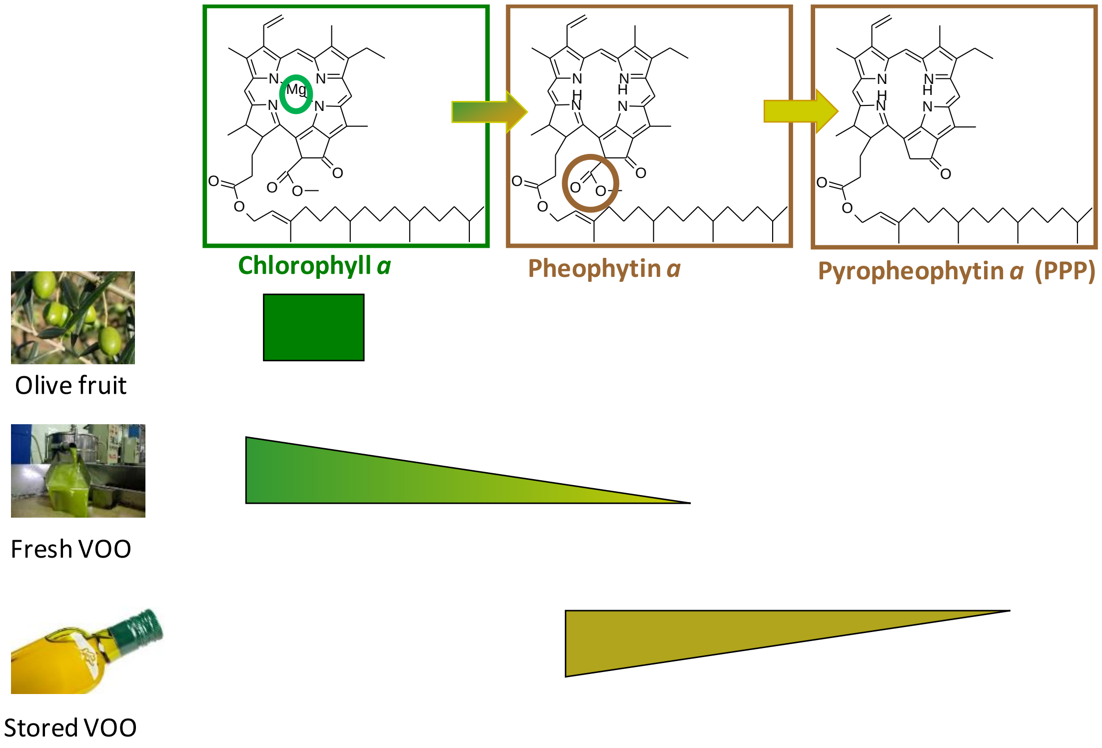
GREX - AGENDA POINT 3.2 EVOLUTION OF PYROPHEOPHYTIN AT LIGHT

The present document is a summary from a yet unpublished study, but distributed in the frame of the GREX OO CHEM meeting. I trust on the confidence of experts and delegates.

The report reveals data arising from basic experimental protocols, and it considers the applicability of a parameter depends on several factors, including non-scientific issues.

1.-Background

Chlorophyll *a* is the main chlorophyll pigment present in higher plants, and therefore in olive fruits, responsible for green coloration. Chlorophyll *a* is a tetrapyrrole coordinated with a central atom of magnesium. In smooth acid conditions (as during the olive oil extraction), the magnesium could be substituted by hydrogen, yielding pheophytin *a*, with brownish coloration. Depending on the intensity of this reaction, virgin olive oils could present high amounts of chlorophylls and lower content on pheophytins (intense green virgin olive oils) or low amounts of chlorophylls and higher content of pheophytins (golden brownish virgin olive oil). During the storage of virgin olive oils, pheophytin *a* could chemically lose a carboxymethyl group ($COOCH_3$ at $C13^2$) originating pyropheophytin *a* (also brownish coloration). Two important points to have in mind are: virgin olive oils contain high or low amount of chlorophylls, but they do always contain pheophytins; the reaction of pyropheophytinization depends on time and temperature.



https://www.ig.csic.es/mariaroca/
mroca@ig.csic.es

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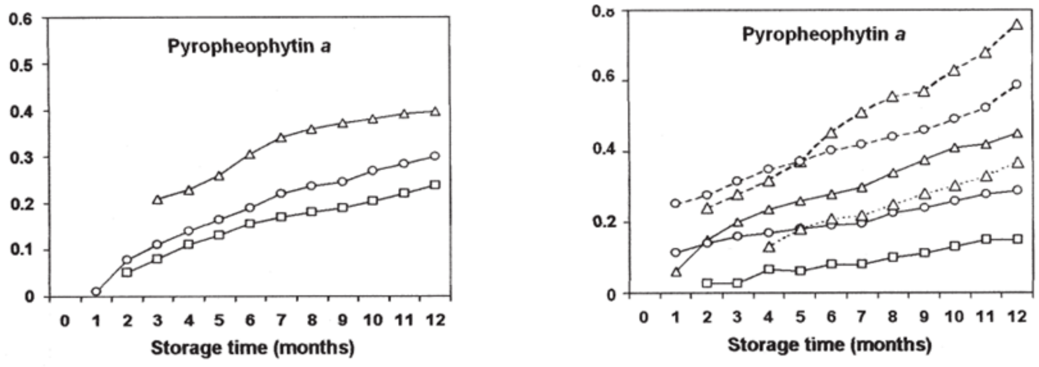
We noted that pyropheophytin *a* increased thoroughly during the dark storage time of virgin olive oils, independently of the fruit variety or initial amount of chlorophylls. We proposed the percentage of pyropheophytin as a storage parameter. After different experiments, we set up the limit of 15% of pyropheophytin *a* for one year in dark, as the VOO is mainly stored in stainless steel tanks.



$$\% \text{ PPP} = \frac{\text{area pyropheophytin } a}{\sum \text{area (pyropheophytin } a + \text{pheophytin } a)} * 100$$

Since then, the pyropheophytin *a* parameter has found supporters and critics. The main critic against this parameter has been its behaviour at light. Therefore, we have developed an assay mimicking the most unfavoured light conditions of a supermarket to study the evolution of the chlorophyll profile in different virgin olive oils under those conditions.

2.-Assay conditions

We selected the most unfavoured conditions forcing the parameter to its limits.

*Raw material: three different old (non-fresh) virgin olive oils were selected: what we called young-old, medium-old and very-old.

*Light intensity: although the European legislation establishes an illumination around 300 lux, for indoor supermarkets, we made our assay at 600 lux with a LED lamp.

*Shadows: we avoided all the shadows coming from neighboring bottles and shelves, as in a typical supermarket, to intensify as maximum the light irradiation conditions.

*Time: the virgin olive oils had a light regime of 12 hours of light/day for 6 days a week.

* Temperature: 21ºC

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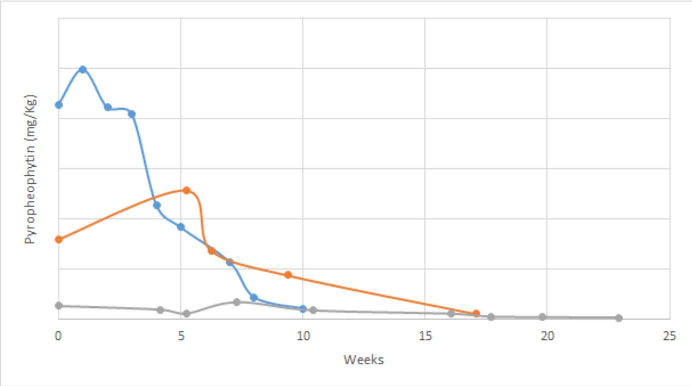
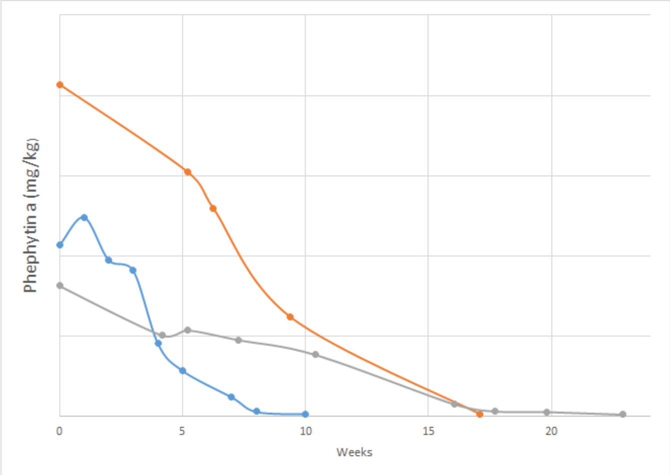
3.-Results

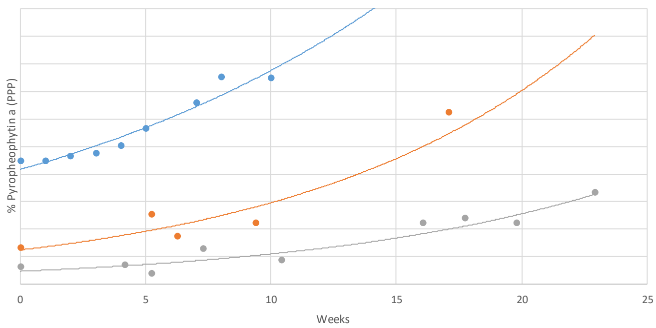
The net content of pyropheophytin *a* (mg/kg) decreased with the storage time at light



However, the kinetics of pheophytin photodegradation is faster than the pyropheophytin *a* in the virgin olive oil



As a consequence, the percentage of pyropheophytin *a* in the virgin olive oil increases with the storage at light



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4.-Discussion

*Effectively, pyropheophytin *a* is degraded under light-stress conditions. However, the percentage of pyropheophytin *a* increases during the light-stress storage conditions, as pheophytin is photobleached faster than pyropheophytin.

*In the light-stress conditions assayed, pyropheophytin is completely degraded at 10-23 weeks of storage. But, before reaching that situation, all the pheophytin content had previously degraded, which is a never seen situation.

*The light-stress conditions required to degrade pyropheophytin are very intense.

5.-Conclusions

*During the dark storage of virgin olive oils (bulk storage in stainless steel tanks, tin or opaque containers), pyropheophytin *a* is an useful storage index as it increases thoroughly with the time.

*During the light storage of virgin olive oils (transparent containers), it is necessary to analyze not only the pyropheophytin *a* content, but other chlorophyll derivatives more sensible to light: pheophytin and/or oxidized chlorophylls.

6.-Questions

1.- In your opinion, what are the possibilities of pyropheophytin *a* to be used as a storage parameter under dark conditions? For example during the olive oil bulk storage in stainless steel tanks.

2.- In your opinion, what are the possibilities of pyropheophytin *a* to be used as a storage parameter under light conditions?

3.- Shall you share your data, if you have it, of pyropheophytin *a* content during the storage of virgin olive oil in whatever storage conditions? The objective could be to compile data subjected as many variables as possible.

4.- If you are developing (or you plan to do it) an olive oil storage experiment, under whatever experimental conditions, would you mind sending me the olive oil samples, so I could analyze the complete chlorophyll fraction by HPLC-*hr*-MS^n?

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mroca@ig.csic.es