OPTIMIZATION OF LIPID HYDROPEROXIDES DETERMINATION USING TPP/TPPO ASSAY COUPLED WITH FTIR-ATR SPECTROSCOPY

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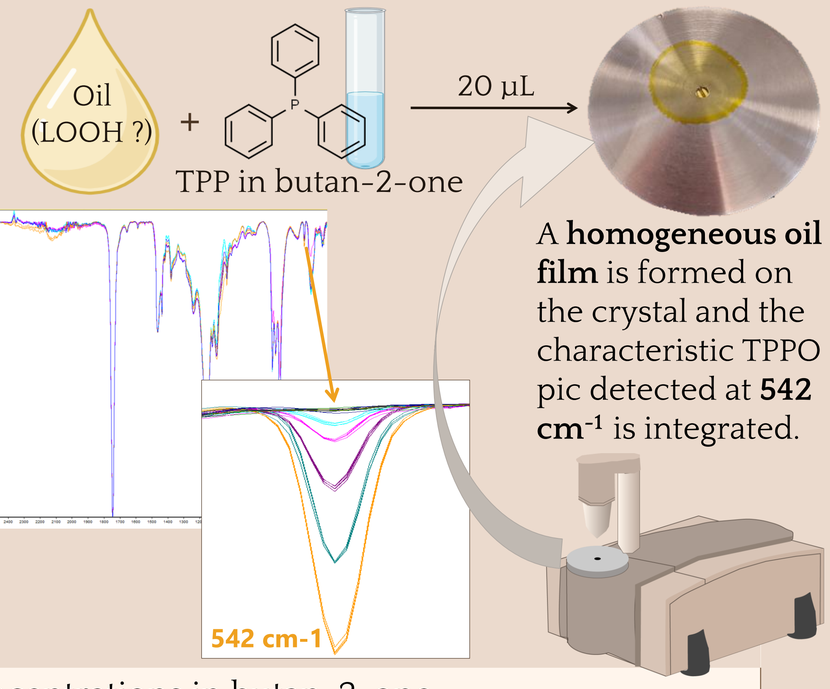
Introduction

Hydroperoxides (LOOH) quantification in oils and fats is a well-used method to determine their degree of oxidation. Iodometric and colorimetric quantification methods are already widely used, but present limitations in particular for highly colored extracts[1]. As a result, alternative method by measuring the oxidation level using **FTIR-ATR** based on the stoichiometric conversion of triphenylphosphine (**TPP**) into triphenylphosphine oxide (**TPPO**) by hydroperoxides is explored[2].

Objective: evaluate applicability of the method on various oils, optimize repeatability and sensitivity limits and study selectivity of dosed hydroperoxides.



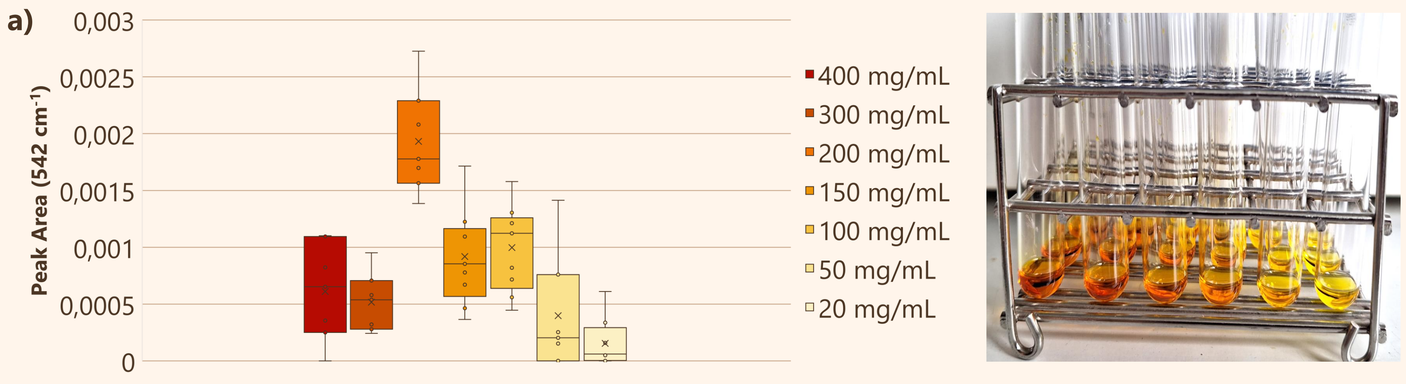
Experimental strategy

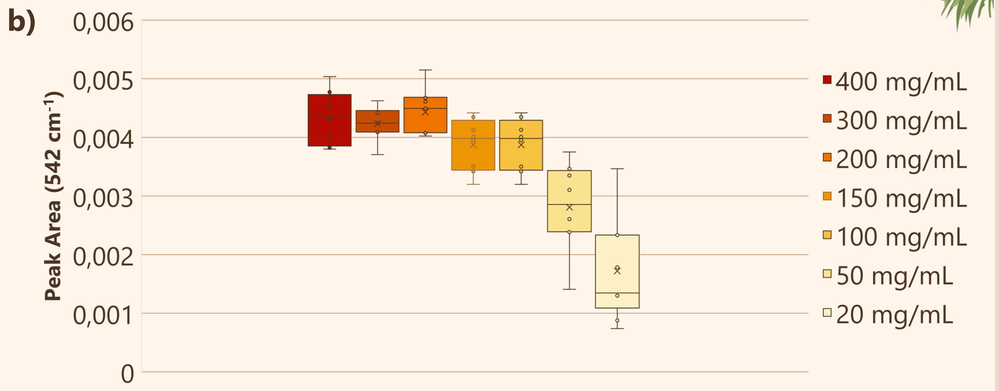
1) Optimizing signal-to-background ratio	Film formation tests at different oil concentrations in butan-2-one. For each concentration, PV values set at **2 mEq02/kg** and **10 mEqO2/kg of oil** with TPPO.
2-3) Sensitivity limit and influence of oil composition	Calibration curves made with different concentrations of TPPO in a mixture butan-2-one/TPP and MCT oil and TPPO in different oils (pigmented, with different degrees of unsaturation and polyphenol content).
4) Tuning of TPP concentration and time of reaction	Measurement of hydroperoxides on oxidized oil using TPP solutions at different concentrations and time.
5) Validation of the stœchiometry of reaction	Comparison of peaks at 542 cm^{-1} from samples of different concentration of TPPO, cumene hydroperoxide, tert-butyl hydroperoxide and hydrogen peroxide in MCT.

Results

1)

Area of peaks at 542 cm^{-1} of different raffia oil concentrations, PV fixed with TPPO at a) 2 mEqO2/kg, b) 10 mEqO2/kg

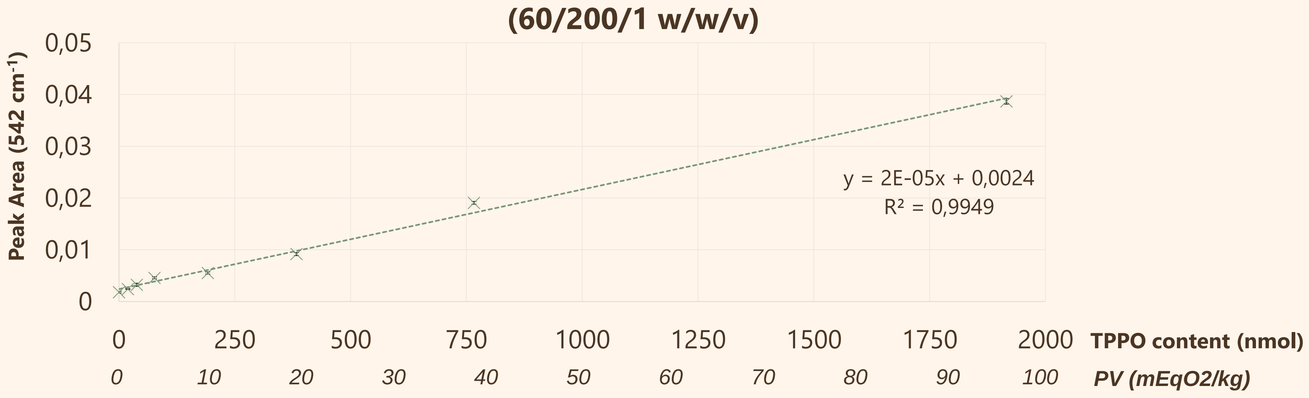




→ The oil concentration of 200 mg/mL provided the highest signal-to-background ratio of TPPO.

2)

Calibration curve of peak area (542 cm^{-1}) as function of TPPO quantities (µmol) and PV (mEqO2/kg) in 200 µL of TPP/MCT/butan-2-one (60/200/1 w/w/v)



→ **PV value of 1 mEqO2/kg is detected**, linear relationship between peak area and amount of LOOH introduced, 200 and 400 mEqO2/kg values also follow this linearity.

3)

VARIABILITY INDEX based on MCT oil peak values 542 cm^{-1} for PV from 20 to 382 mEq O2/kg	**Menhaden oil**	**Olive oil**	***Phaeodactylum tricornutum* extract**	**Raffia oil**
	4,27 ± 1,37 %	3,73 ± 3,08 %	6,46 ± 4,63 %	6,16 ± 3,81 %
	Rapeseed oil	**Rapeseed oil + 2500 ppm of fucoxanthine**		**Walnut oil**
	5,41 ± 1,93 %	3,66 ± 1,73 %		3,41 ± 2,93 %

→ Oil composition only slightly influences the signal response (variation ≤10%). Building a TPPO calibration curve for each oil may be recommended to increase accuracy.

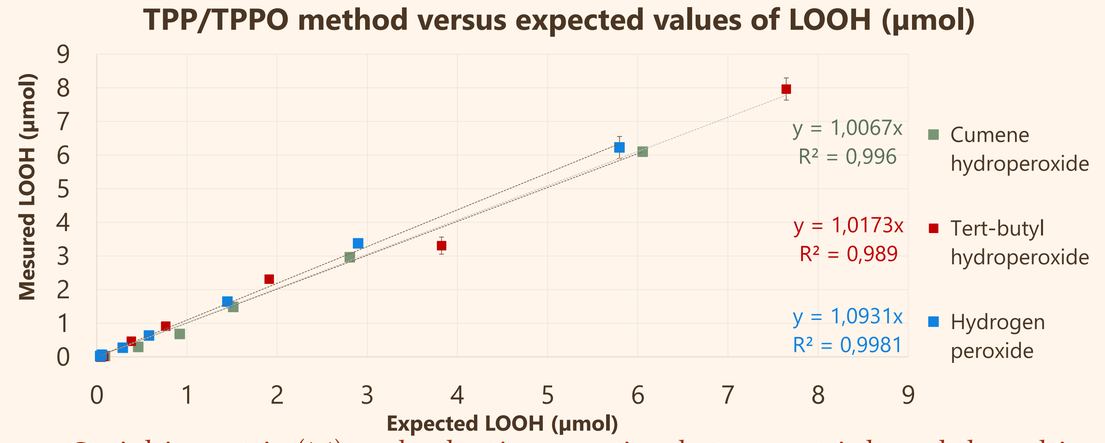
4)

PV (mEqO2/kg) dosed at different concentrations of TPP and time

→ No significative difference as long as TPP is introduced in excess. Increases of peak area after 17 hours, proportional to the concentration of the TPP solution, realizing assay in the hour is recommended.

5)

Mesured LOOH (µmol) from peroxides added in MCT oil via TPP/TPPO method versus expected values of LOOH (µmol)



→ Stoichiometric (1:1) and selective reaction between triphenylphosphine (TPP) and hydroperoxides.

Conclusion

This rapid method offers great promise in terms of reproducibility and robustness of results, with absolute quantification of hydroperoxides. Furthermore, the assay is not affected by highly colored extracts (containing chlorophyll in particular). The optimization of the TPP/TPPO method's sensitivity enabled LOOH detection at the nanomole range.

References

[1] Hornero-Méndez, D.; Pérez-Gálvez, A.; Mínguez-Mosquera, M. I. A Rapid Spectrophotometric Method for the Determination of Peroxide Value in Food Lipids with High Carotenoid Content. *J. Am. Oil Chem. Soc.* **2001**, *78* (11), 1151–1155.

[2] Deyrieux, C.; Villeneuve, P.; Baréa, B.; Decker, E. A.; Guiller, I.; Michel Salaun, F.; Durand, E. Measurement of Peroxide Values in Oils by Triphenylphosphine/Triphenylphosphine Oxide (TPP/TPPO) Assay Coupled with FTIR-ATR Spectroscopy: Comparison with Iodometric Titration. *Eur. J. Lipid Sci. Technol.* **2018**

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