Silvana Alfei, Paolo Oliveri, Cristina Malegori, Eleonora Mustorgi

A NANOSPHERICAL DENDRIMERIC GALLATE ESTER FOR LONG TERM PRESERVATION OF ESSENTIAL OILS: AN INTEGRATED CHEMOMETRIC ASSISTED FT-IR STUDY

Università di Genova – DIFAR Dipartimento di Farmacia. Viale Cembrano, 4 – 16148 Genova (GE), Italy
mustorgi@difar.unige.it

1. INTRODUCTION

Essential oils (**EOs**) are hydrophobic liquids from plants made of volatile and easily oxidizable chemical compounds. **EOs** are very popular in the food, cosmetic and pharmaceutical industry as aromas, fragrances and alternative therapeutic devices,[1,2] but their susceptibility to oxidative degradation, triggered by temperature, light and oxygen, limits their shelf life and causes loss of quality, alterations of sensory and pharmacological properties with production of smelly or even harmful compounds, responsible for allergic reactions and skin irritation.[3-5] Synthetic preservatives are commonly adopted but with limited efficiency and danger of health diseases.[6] Natural polyphenols as gallic acid (**GA**) are nowadays proposed as safer alternatives, but their efficiency is limited by their low compatibility with lipids and possible pro-oxidant actions. Recently, a hydrophobic, biodegradable and not cytotoxic **GA**-enriched dendrimer (**GAD**) characterized by a nanospherical morphology (**Fig. 1**) and endowed with a remarkable antioxidant activity (**Fig. 2**) was synthetized according to **Scheme 1.**[7] Why not try to use it to prevent oxidative degradation of **EOs** (**Fig. 3**) in place of synthetic preservatives or the free form of **GA**???

2. MORE IN DEEP CONCERNIG GAD…..

Further studies recently completed and close to publication, have shown that **GAD**, with respect to free **GA**, possesses also more efficient antibacterial properties against several antibiotics-resistant G+ strains, inhibits platelet aggregation and ROS accumulation.[8] Consequently, its adding to **EOs** with the aim at preventing their spoilage and extending their shelf life, also the **EOs** intrinsic pharmacological properties, would be in turn improved.

3. IN THIS STUDY

In this work, **GAD** was investigated to evaluate its activity as additive to prevent, contrast or postpone the naturally occurring oxidative degradation of **EOs** and its efficiency was compared to that of **GA**. Low cost, simple, rapid, conservative and reproducible **FTIR** spectroscopy was adopted as a leading evaluation method for analyzing the samples under study, identifying the less degraded ones and tracking down the most suitable additive between **GAD** and **GA**. The **FTIR** spectral data were analyzed both by studying the changes in the areas ratio of bands identified as indicators of degradation in function of oxidation time (**BAR**) and by multivariate analysis (**MVA**). Iodometric titration for determining the peroxide value (**PV**) was performed for confirmation of the FTIR results.[9]

Scheme 1

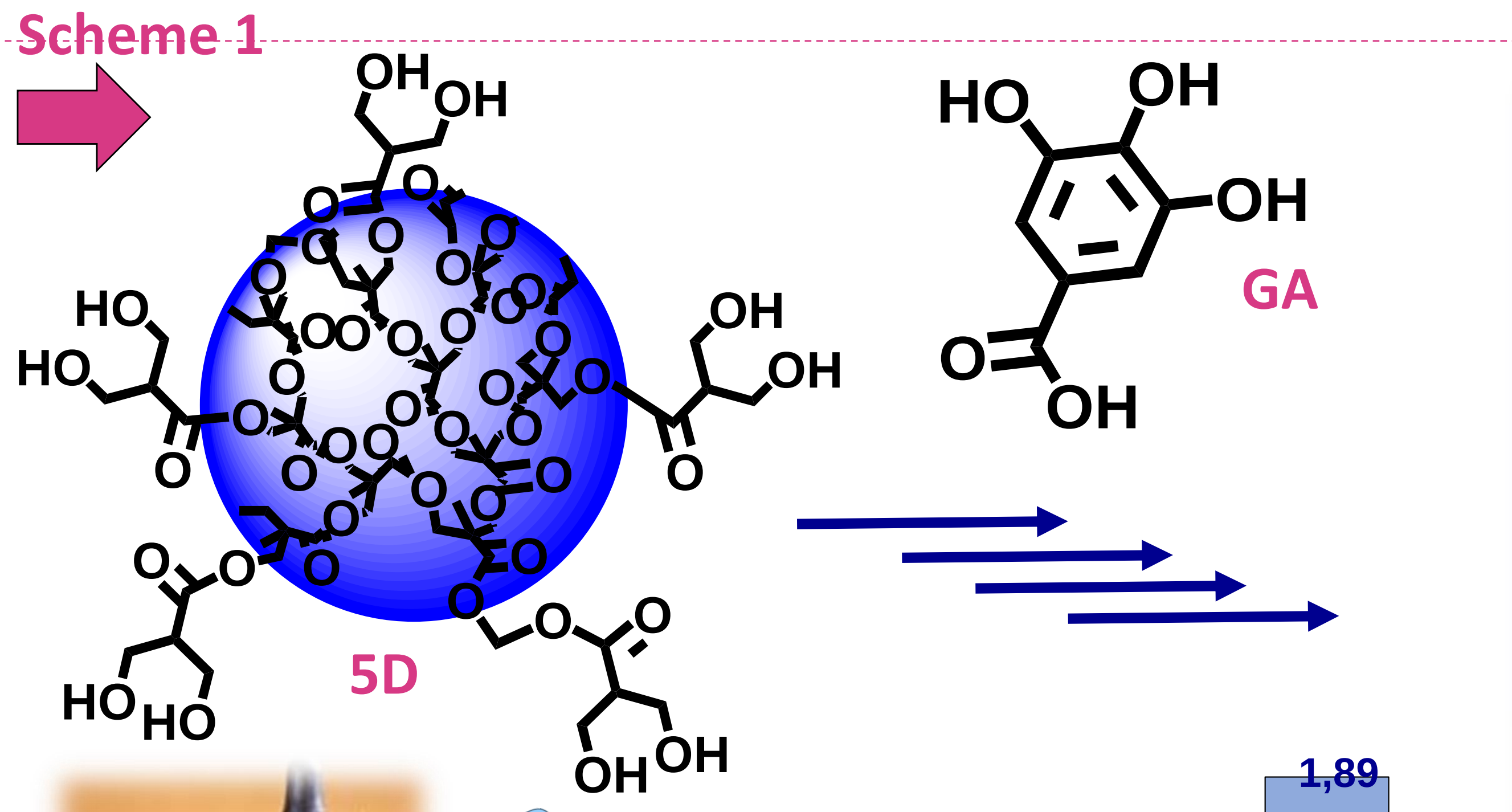


Figure 3. *Pinus Mugo* essential oils

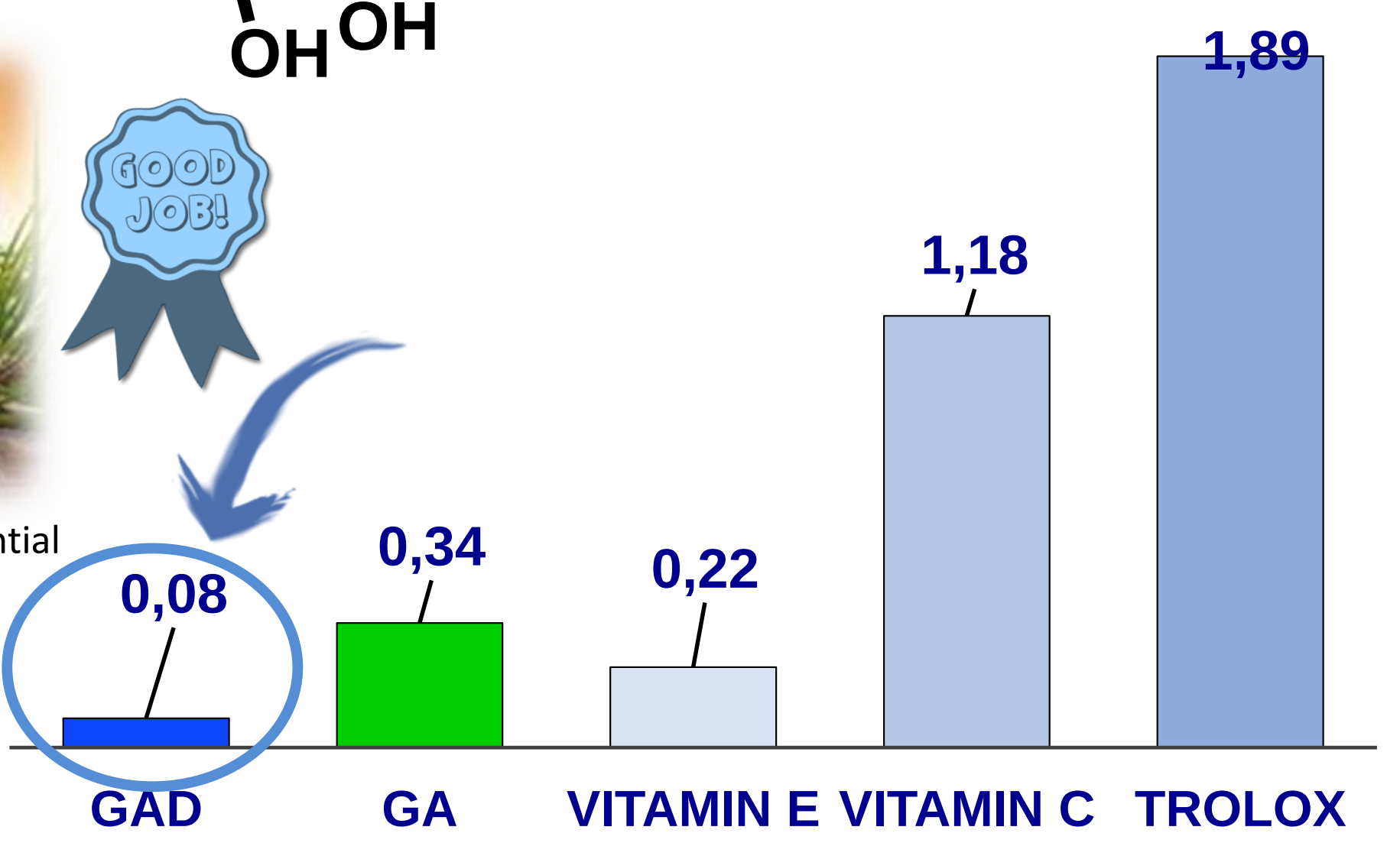


Figure 2. Comparison between the RSA expressed as IC_{50} mM of GAD, GA, Vit. C, E and Trolox.

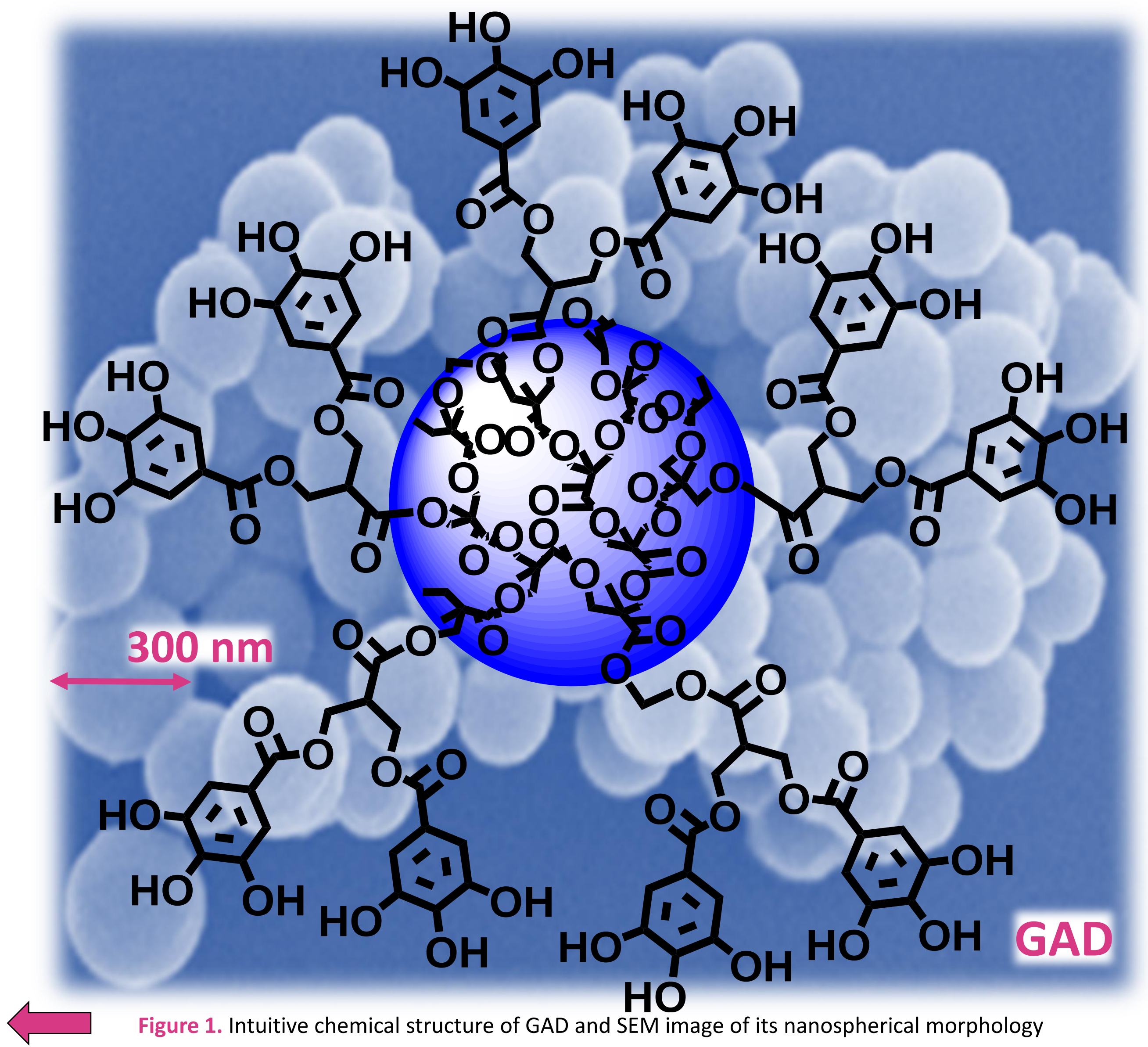


Figure 1. Intuitive chemical structure of GAD and SEM image of its nanospherical morphology

4. THE PROJECT

The study was firstly performed on a single reference compound commonly present in EOs as a simplified model systems, then on the whole EO. The single component was ***β-pinene*** (***β-P***) that, being Nopinone the main product coming from its oxidation, offered the possibility to identify and track easily the changes that occurred in the FTIR bands during the degradative process (**Fig. 4**). The oil was ***Pinus Mugo*** EO (**PEO**) because rich in pinene chosen as model.

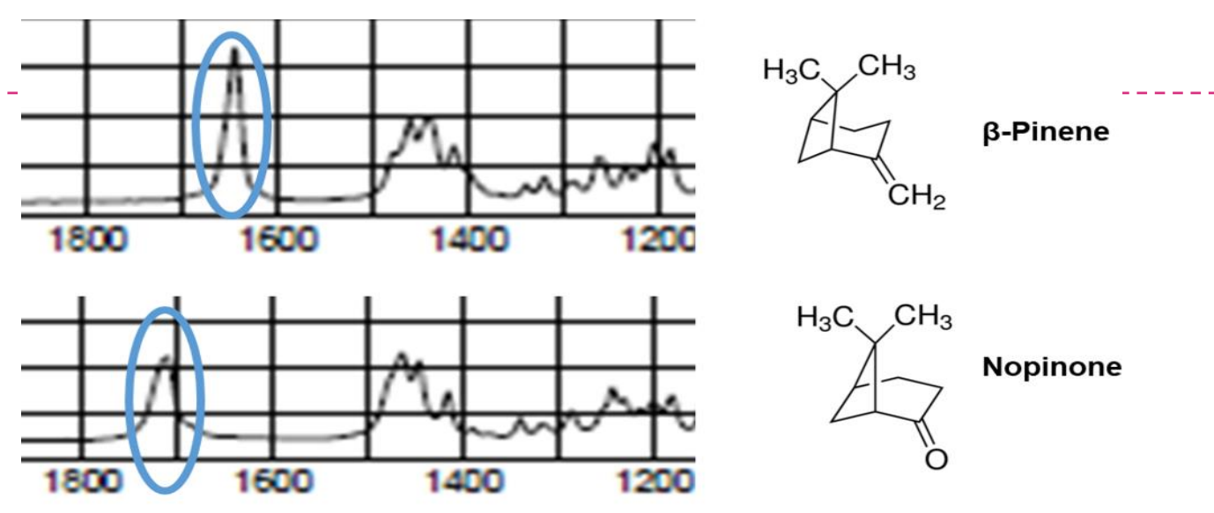
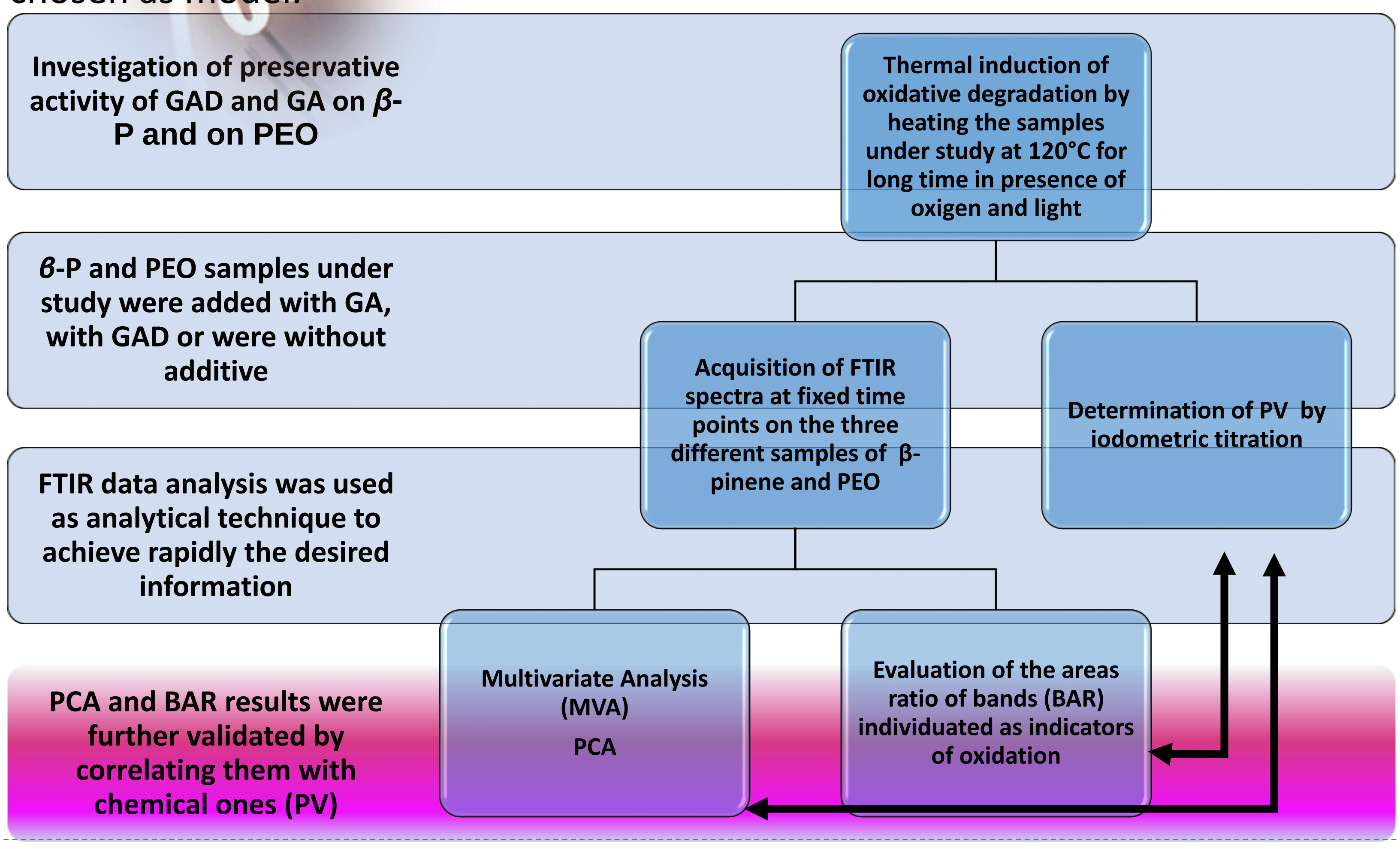


Figure 4. Main product of oxidation of *β*-P (Nopinone) and changes occurring in FTIR spectrum.



Investigation of preservative activity of GAD and GA on *β*-P and on PEO

Thermal induction of oxidative degradation by heating the samples under study at 120°C for long time in presence of oxigen and light

β-P and PEO samples under study were added with GA, with GAD or were without additive

Acquisition of FTIR spectra at fixed time points on the three different samples of β-pinene and PEO

Determination of PV by iodometric titration

FTIR data analysis was used as analytical technique to achieve rapidly the desired information

Multivariate Analysis (MVA)
PCA

Evaluation of the areas ratio of bands (BAR) individuated as indicators of oxidation

PCA and BAR results were further validated by correlating them with chemical ones (PV)

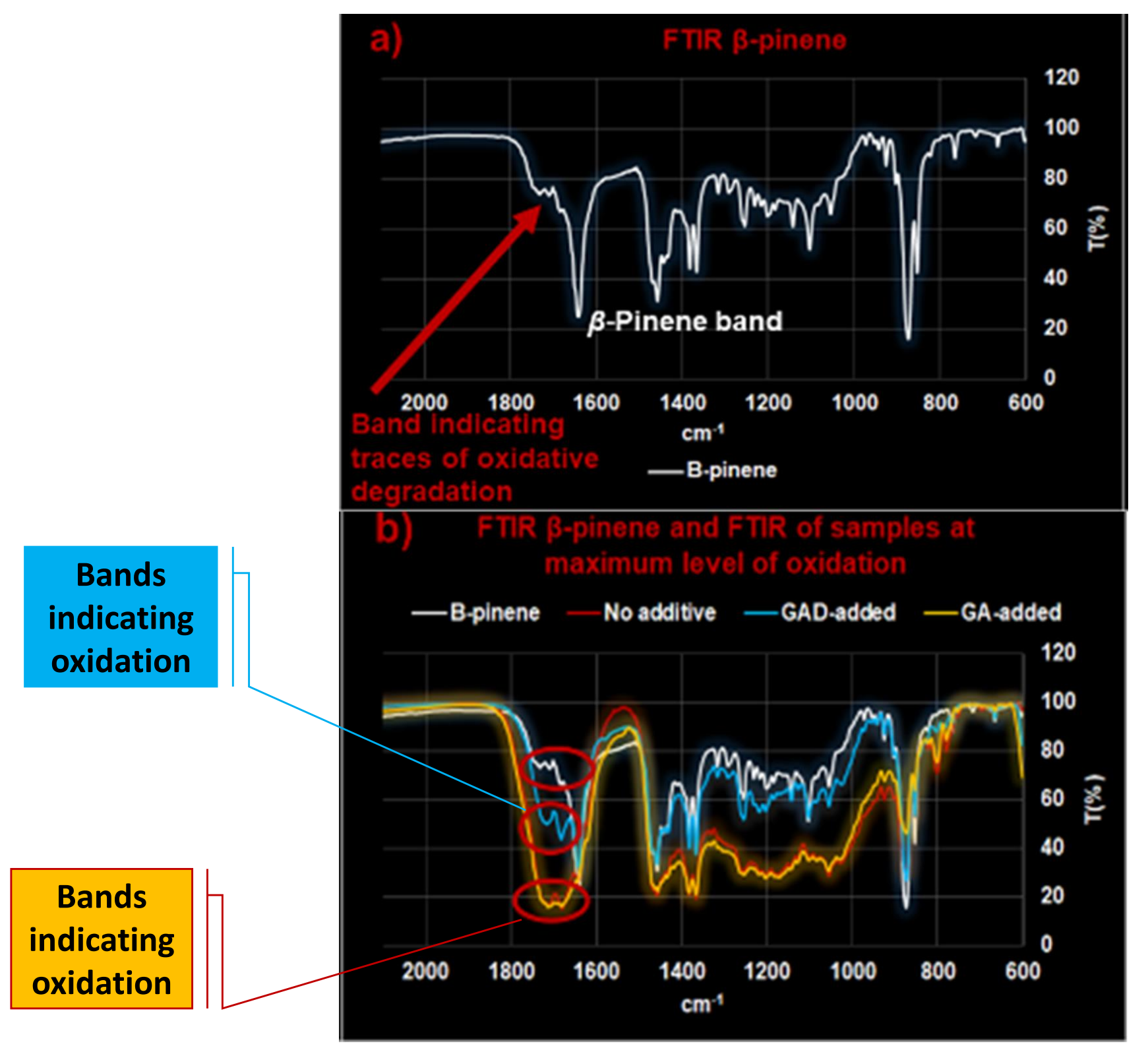


Figure 5. FTIR spectrum of non heated *β*-P (**a** and **b**) and of the three samples under study at maximum rate of oxidation (**b**): *β*-P (orange line), *β*-P+**GA** (red line), *β*-P+**GAD** (blue line).

5. RESULTS

***β*-Pinene**. By observing the relative intensity of significant bands (1642-1750 cm^{-1}) in the FTIR spectra of the samples under study (**Fig. 5.b**), it appears that, when **GAD** was added, the degradation resulted strongly inferior and blocked at early stage than in the case of the sample without additive or added with **GA**. This information precisely matches the response obtained from **MVA** and observable in the **PCA** score plot (**Fig. 6.a**). In **PC1**, the progressive degree of oxidative degradation can be detected proceeding from left to right. Loading profiles of **PC1** (blue line in **Fig. 6.b**), assert that the degradation phenomena can be followed thanks to a decrease of the FTIR band at 1642 cm^{-1} and a simultaneous increase of the spectral features between 1700 and 1750 cm^{-1} as visible in **Fig. 5.b**. By considering the area ratio of these bands as function of heating time (**BAR** method), the oxidative degradation profile for the three samples investigated was also obtained, which confirmed the **MVA** results. Both **MVA** and **BAR** outcomes were furtherly confirmed by peroxide value evaluation.

PEO. Concerning the more complex system of the hole EO, the progressive oxidative degradation in **PCA** score plot, can be observed in **PC2** going from bottom to top (**Fig. 7**). In the samples containing **GAD**, the oxidation was blocked at the initial stages (**red square**), while the addition of **GA** translated in a limited conservative action and, for prolonged heating times in a pro-oxidative action (**green square**), improving spoilage rather than limiting it. This outcome was confimed also by data obtained analyzing FTIR spectra with **BAR** (**Fig. 8**) method and by **PV** (**Fig. 9**).

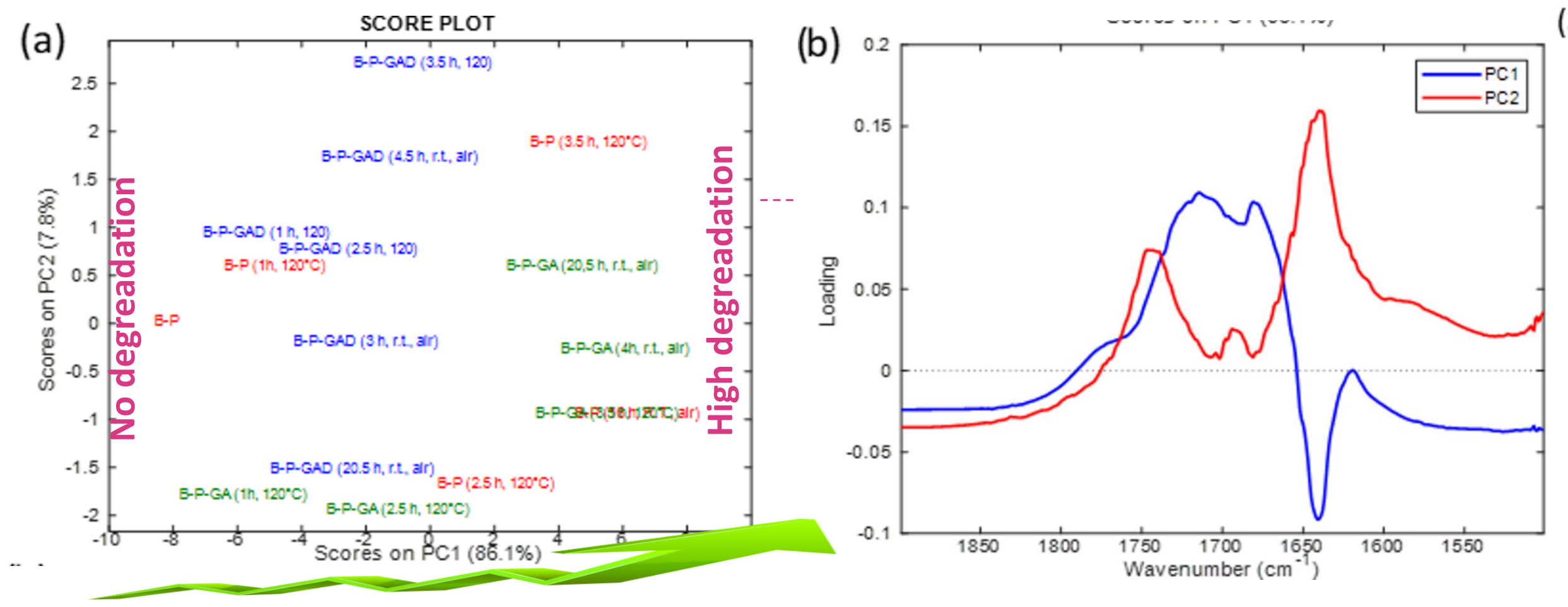


Figure 6. PCA score plot and loading plot concerning β-P. PCA score plot: PC1 vs. PC2. β-P samples in red, **GA**-added samples in green, **GAD**-added samples in blue (**a**); PCA loading plot: PC1 versus PC2 (**b**).

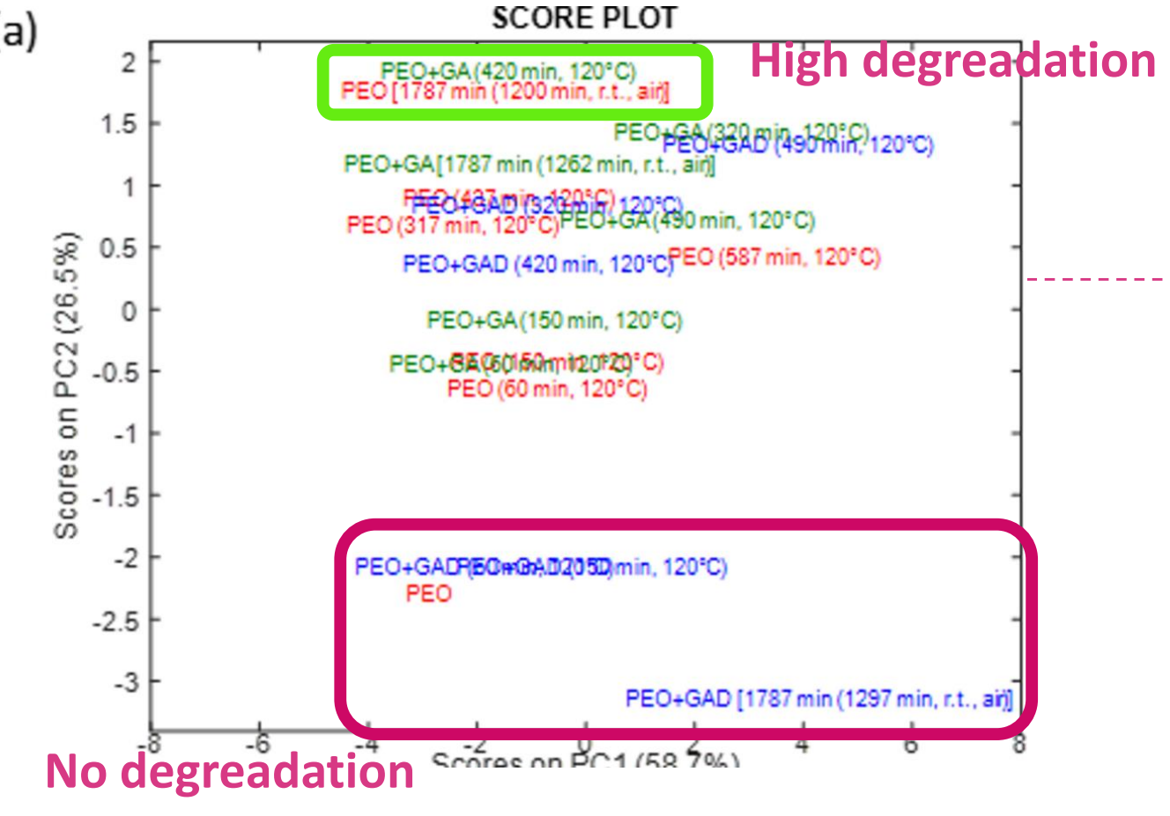


Figure 7. PCA score plot concerning PEO. PCA score plot: PC1 vs. PC2. PEO samples in red, **GA**-added samples in green, **GAD**-added samples in blue.

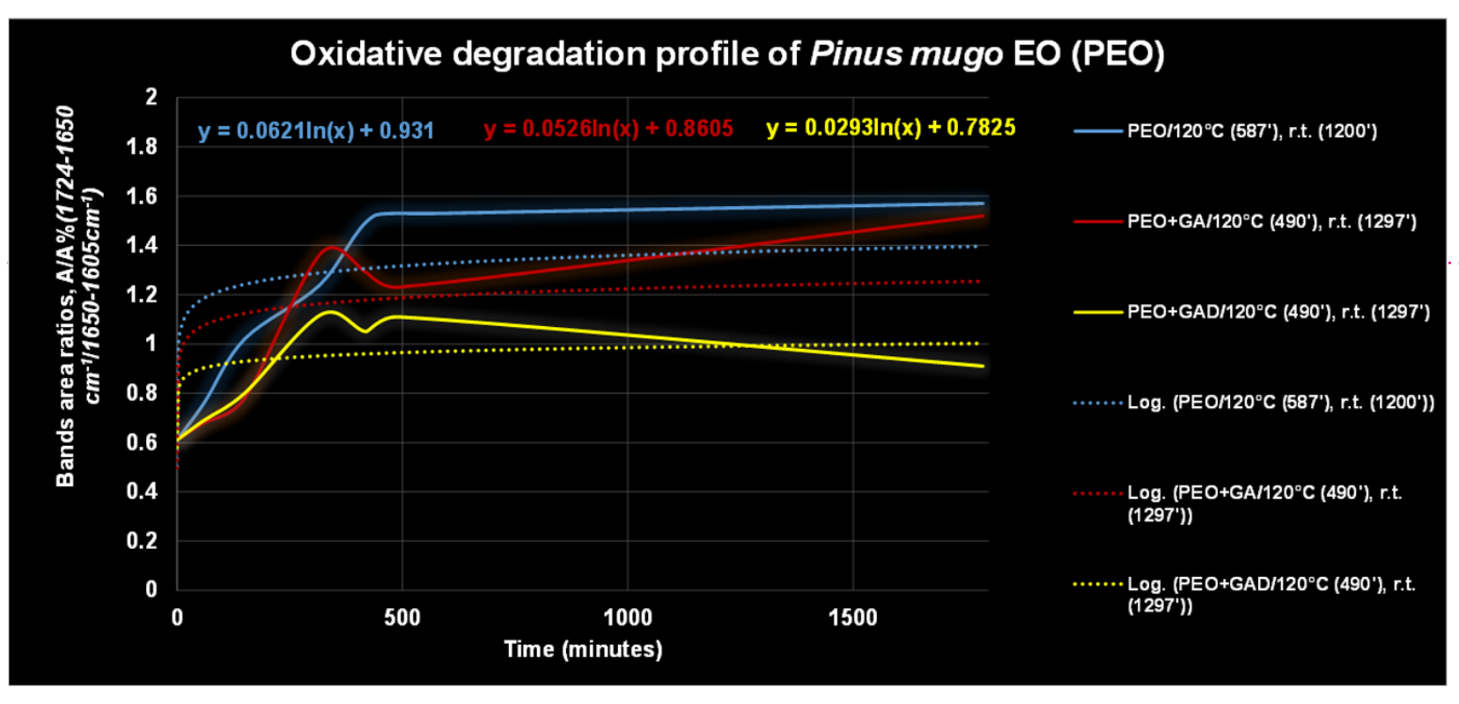


Figure 8. PEO oxidative degradation profiles as FTIR bands areas ratio (**BAR**)(range 1700-1506 cm-1) in function of time .

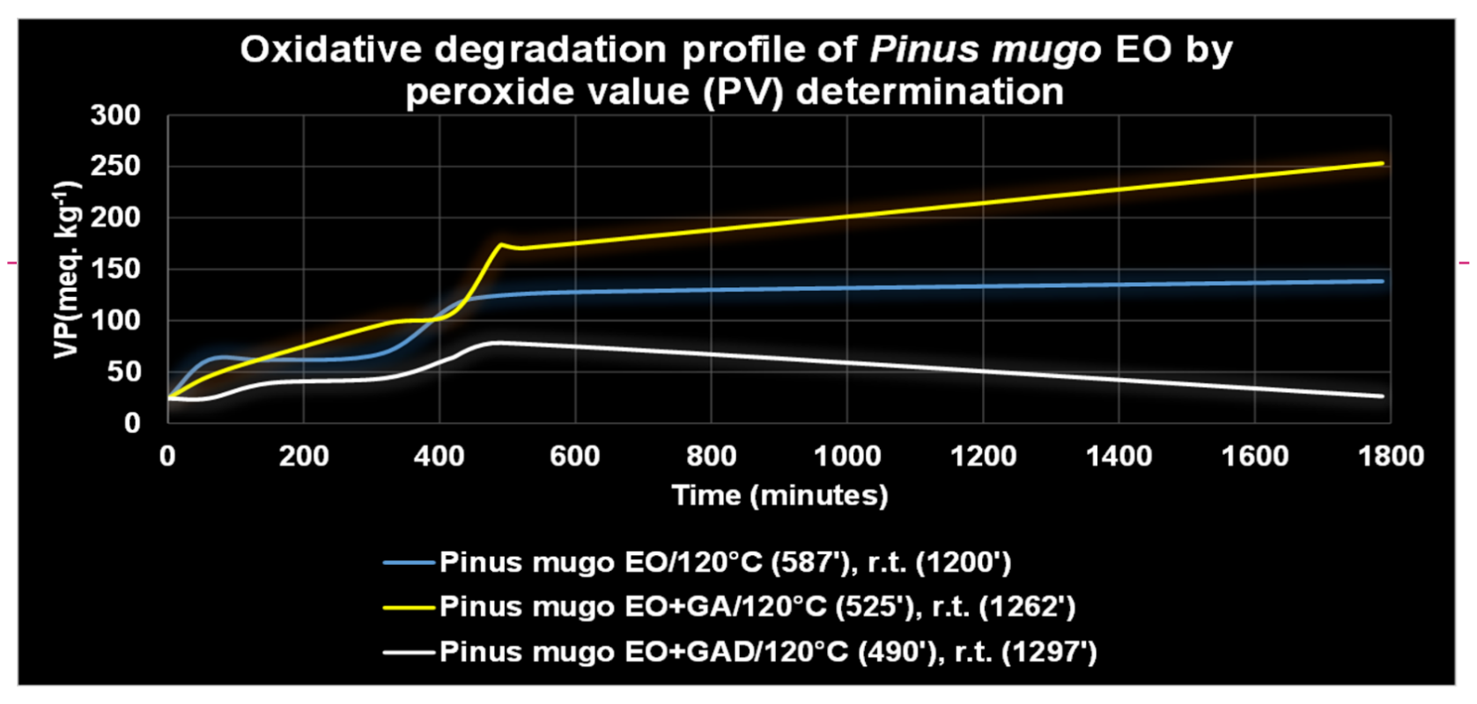


Figure 9. Peroxide value (PV) determined by iodometric titration of PEO along thermally-induced oxidative degradation.

CONCLUSIONS

By merging FTIR analysis, processed by MVA and BAR methods and chemical investigations, the efficiency of a spherical dendrimer nanomaterial peripherally esterified with sixty-four **GA** moieties (**GAD**) in preventing essential oils spoilage, was investigated and compared to that one of **GA**. The study was developed on ***Pinus Mugo*** essential oil. All the adopted analytical techniques provided comparable answers asserting that **GAD** is far more efficient than **GA**, it never acts as pro-oxidant and can be considered an innovative nanospherical material, with strong potentials for being applied as preservative in lipid substrates.

References: [1] Yamamoto S., SOFW J., 2008, 134, 8. [2] Jiang Y., Wu N., Fu Y.-J., Wang W., Luo M., Zhao C.-J., Zu Y.-G., and Liu Y.-L., Environ. Toxicol. Pharmacol., 2011, 32, 63. [3] Hagvall L., Skold M., Brared-Christensson J., Borje A., and Karlberg A.-T., Contact Dermatitis, 2008, 59, 143. [4] Skold M., Hagvall L., and Karlberg A.-T., Contact Dermatitis, 2008, 58, 9. [5] Brared-Christensson J., Matura M., Gruvberger B., Bruze M., and Karlberg A.-T., Contact Dermatitis, 2010, 62, 32. [6] Hirose M., Takesada Y., Tanaka H., Tamano S., Kato T., and Shirai T., Carcinogenesis, 1998, 19, 207. [7] Alfei S., Catena S., and Turrini F., Drug Deliv. Trans. Res., under review. [8] Alfei S., Signorello M. A., Schito A., Catena S., and Turrini F., results not yet published. [9] Alfei S., Oliveri P., and Malegori C., ChemistrySelect, under review.

DIPARTIMENTO DI FARMACIA
UNIVERSITÀ DI GENOVA

UNIVERSITÀ
DEGLI STUDI DI BARI
ALDO MORO

XXVIII Congress of the Analytical Chemistry Division
Bari, 22-26 September 2019

Società Chimica Italiana
Divisione di Chimica Analitica

DIFAR