FunTomP

FunctionalizedTomatoProducts

EPR SPIN TRAPPING
-ADVANTAGES AND DISADVANTAGES-
FOR PRODUCTS WITH HIGH ANTIOXIDANT CAPACITY

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This project has received funding from the European Union's PRIMA programme under grant agreement No 2032

PRIMA
PARTNERSHIP FOR RESEARCH AND INNOVATION IN THE MEDITERRANEAN AREA

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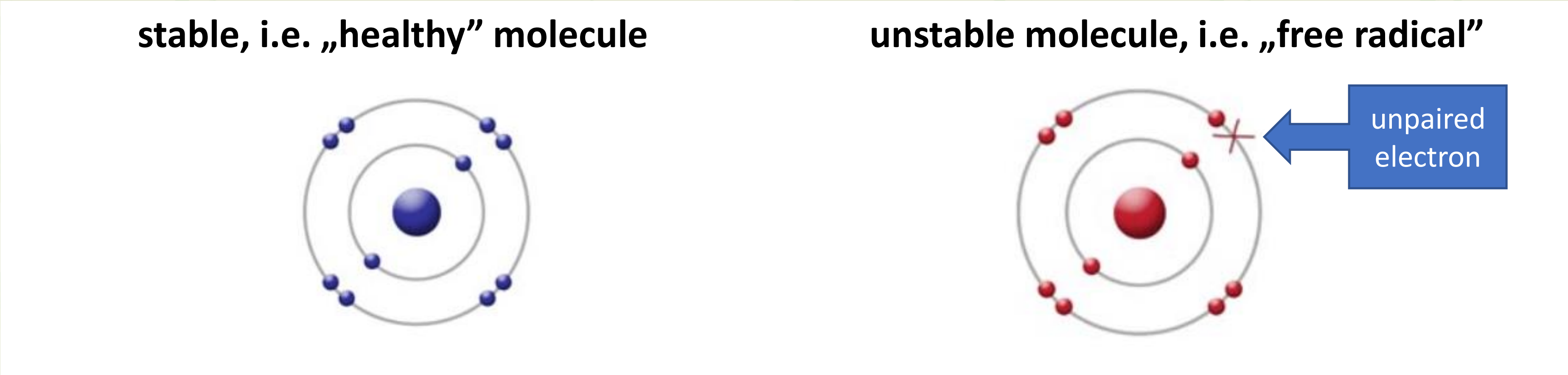
WHAT IS EPR?

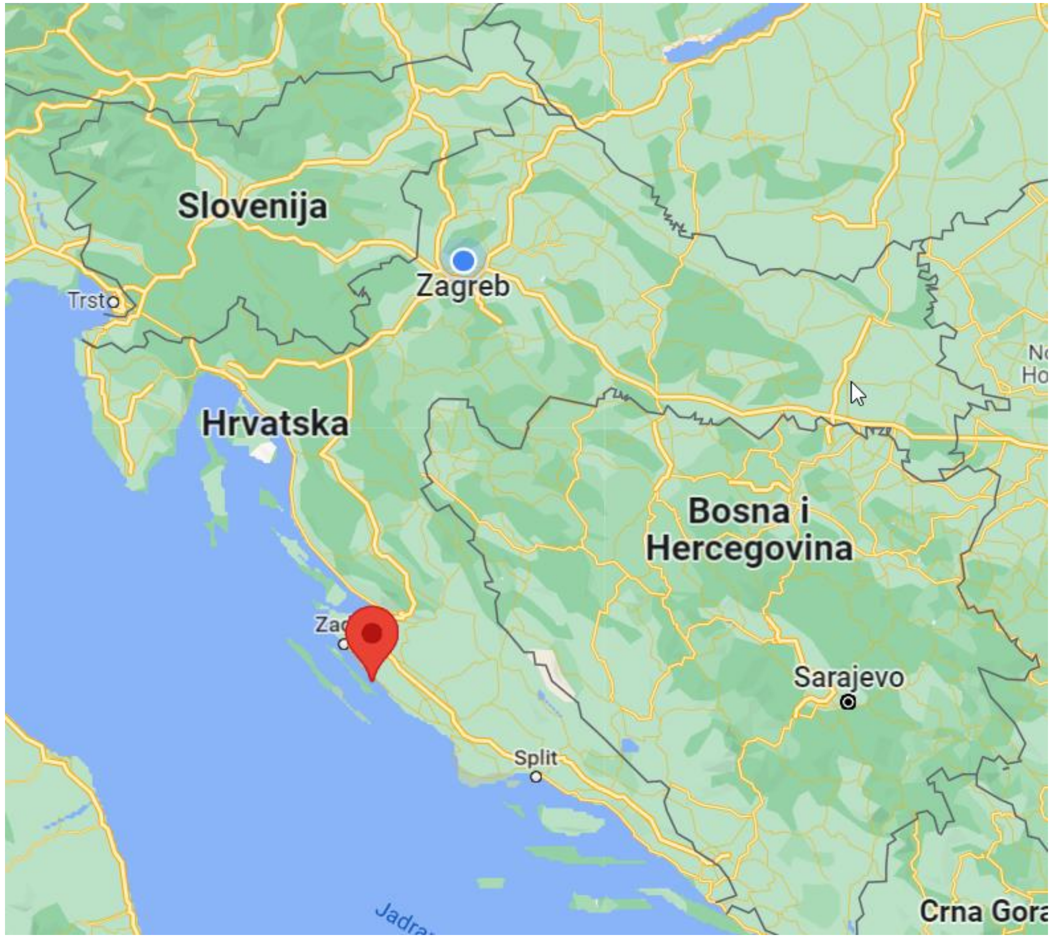
ELECTRON PARAMAGNETIC RESONANCE (EPR)
OR
ELECTRON SPIN RESONANCE (ESR)
OR
ELECTRON MAGNETIC RESONANCE (EMR)

- Spectroscopy - magnetic resonance technique that detects *UNPAIRED ELECTRONS* (transition metal ions (Fe, Cu, Mn, Co, Ni....); free radical – typically species with O, N, C; defects – semiconductors, light, radiation induced etc.)



- only technique that unambiguously detects *FREE RADICALS* - no possibility of false positive results

Tomato Harvesting October, 2021



Mediterranean Diet

Tomato (*Solanum lycopersicum*)

Juices & Sauces

Croatian Eco Farming

https://holus.hr/

VRTNI CENTAR BAKOVIĆ

SVETI FILIP I JAKOV

VRTNI CENTAR Bakovic

EPR EQUIPMENT

- Electron paramagnetic resonance (EPR) measurements were carried out on a benchtop Bruker Magnettech ESR5000 spectrometer (Bruker BioSpin, Germany) equipped with liquid nitrogen variable temperature controller TCH04 (Bruker BioSpin, Germany).

- Measurement parameters were as follows: microwave frequency at 9.5 GHz, magnetic field modulation frequency at 100 kHz, and magnetic field modulation amplitude at 0.1 mT.

Bruker benchtop EPR Spectrometer Magnettech ESR5000

- Microwave frequency 9.2-9.6 GHz (X-band)
- **Sensitivity of $5x10^{10}$ spins/mT**
- Concentration sensitivity 50 pM
- Signal-to-noise-ratio S/N 600:1
- Digital resolution 32 bits
- Magnet resolution 0.03 µT (0.3 mG)
- Magnetic field range 0-650 mT
- Stability 1.0 µT/h (10 mG/h)
- Temperature range -80-200 °C
- Weight cca. 45 kg

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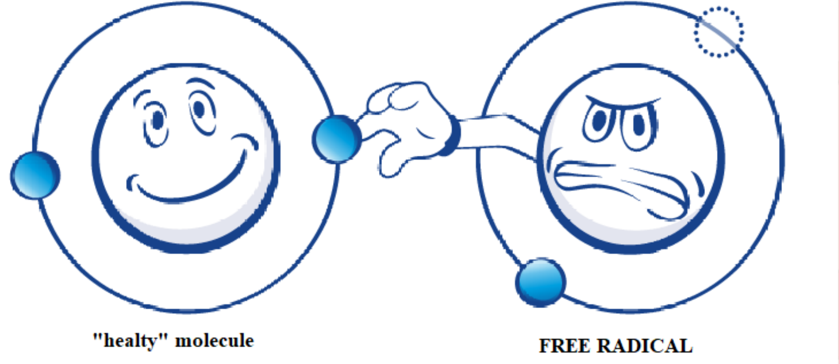
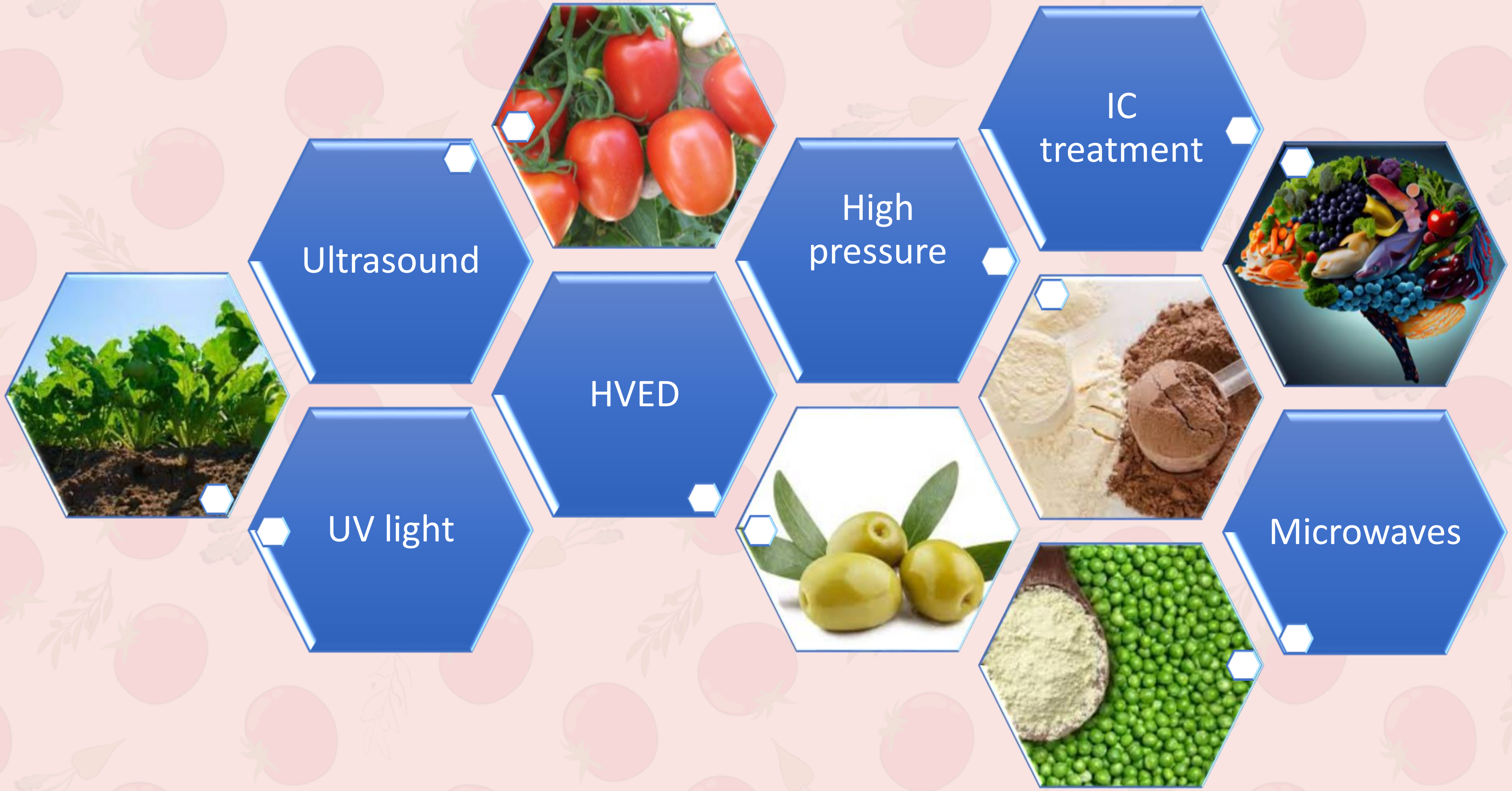
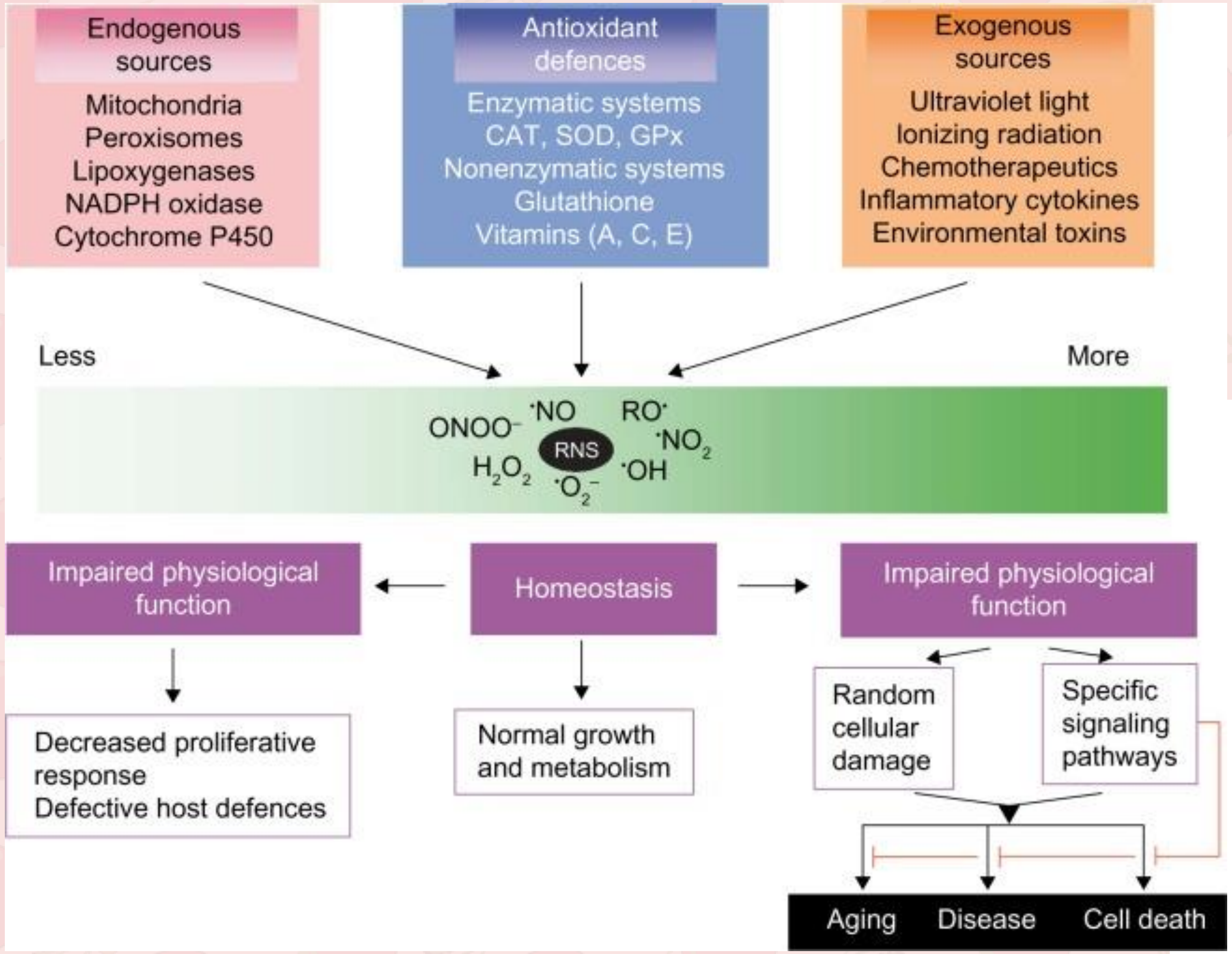
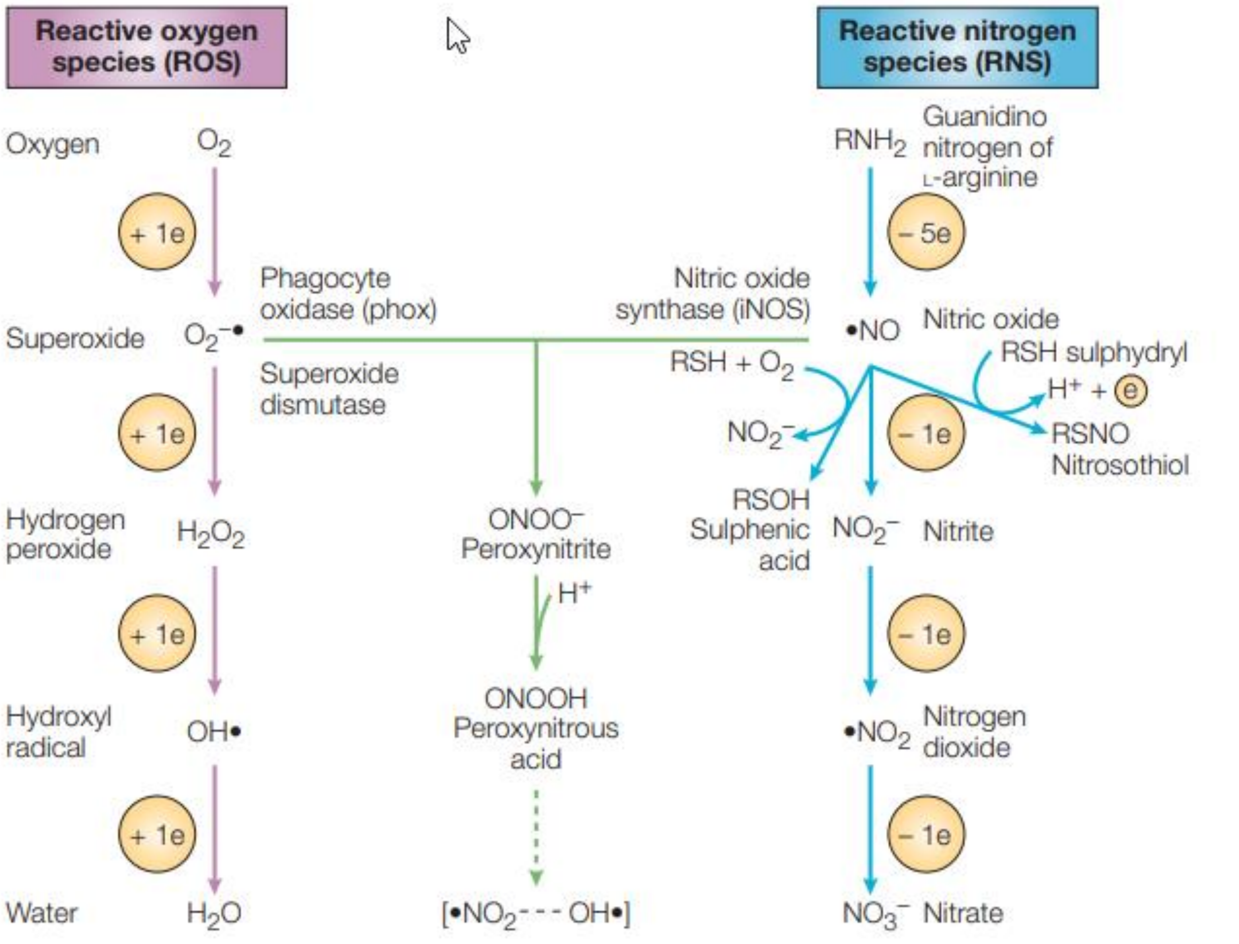
INDUCTION AND MONITORING RADICALS IN SAMPLES/PLANTS







Fang FC. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. Nat Rev Microbiol. 2004 Oct;2(10):820-32. doi: 10.1038/nrmicro1004



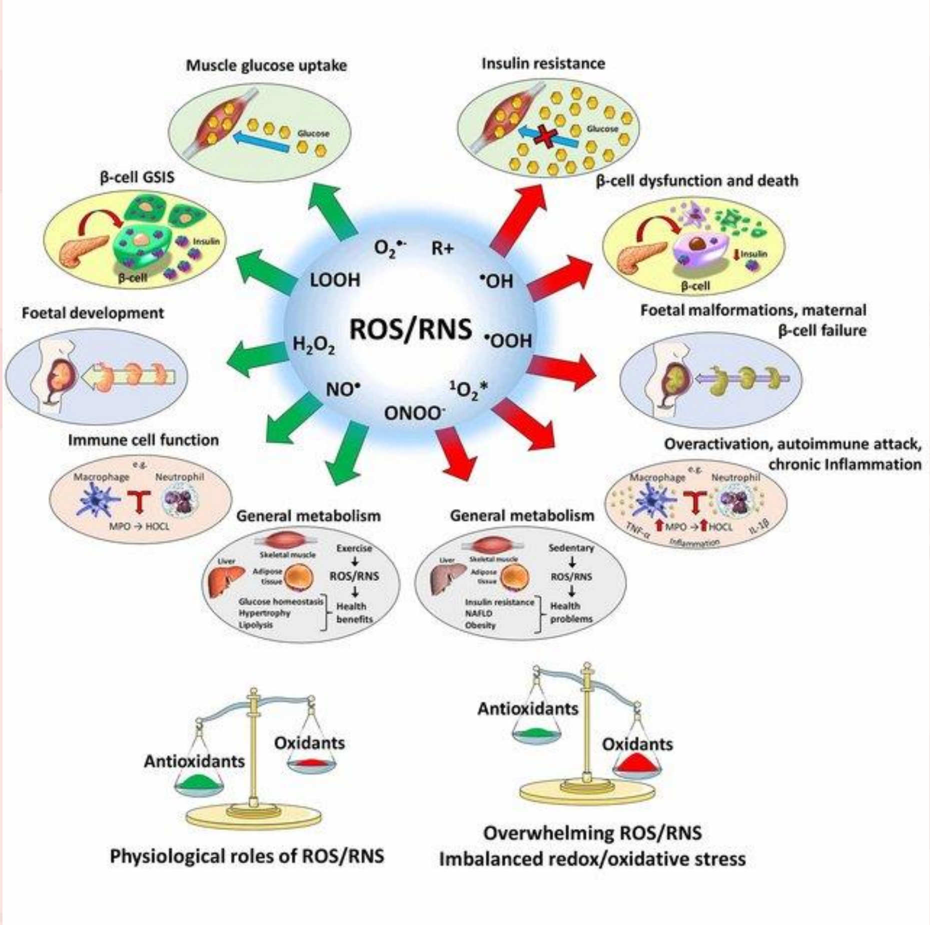
Domej W, Oettl K, Renner W. Oxidative stress and free radicals in COPD--implications and relevance for treatment. Int J Chron Obstruct Pulmon Dis. 2014 Oct 17;9:1207-24. doi: 10.2147/COPD.S51226

Name	Formula	Characteristics
Hyperoxide/ superoxide	$^{\cdot}O_2^-$	Highly unstable, signaling function, synaptic plasticity
Hydrogen peroxide	H_2O_2	Cell toxicity, signaling function, generation of other ROS
Hydroxyl radical	$^{\cdot}OH$	Free radical, highly unstable, very reactive agent
Alkoxyl radical	$RO^{\cdot}$	Free radical, reaction product of lipids
Peroxyl radical	$ROO^{\cdot}$	Free radical, reaction product of lipids
Hypochlorite anion	OCl^-	Reactive oxygen species, reactive chlorine species, enzymatically generated by myeloperoxidase
Singlet oxygen	$^1O_2$	Induced/excited oxygen molecule, radical and nonradical form
Ozone	O_3	Environmental toxin
Nitric oxide	$^{\cdot}NO$	Environmental toxin, endogenous signal molecule
Peroxynitrite	$ONOO^-$	Highly reactive reaction intermediate of $^{\cdot}O_2$ and $^{\cdot}NO$
Nitrogen dioxide	$^{\cdot}NO_2$	Highly reactive radical, environmental toxin
Nitrogen oxides	NO_x	Environmental toxins, including NO and $^{\cdot}NO_2$, derived from the combustion process

Abbreviations: RNS, reactive nitrogen species; ROS, reactive oxygen species.

DISADVANTAGES

- very reactive
- very unstable
- short lifetime
- considerable damage to the cells

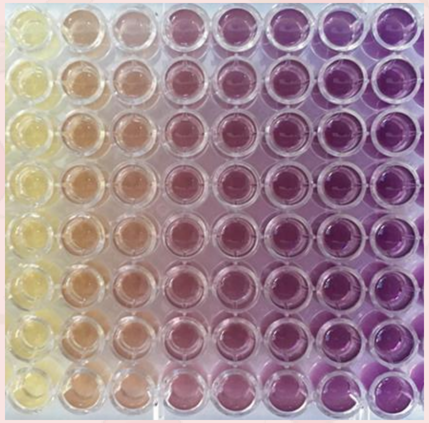


EPR SPECTROSCOPY
IMPORTING OR MONITORING RADICALS IN SAMPLES/PLANTS

IMPORTING RADICALS IN SAMPLES

FUNCTIONALITY

like ANTIOXIDANT ACTIVITY



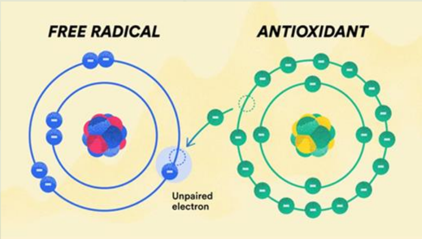
SPIN TRAPPING

MONITORING RADICALS TRAPED IN SAMPLES

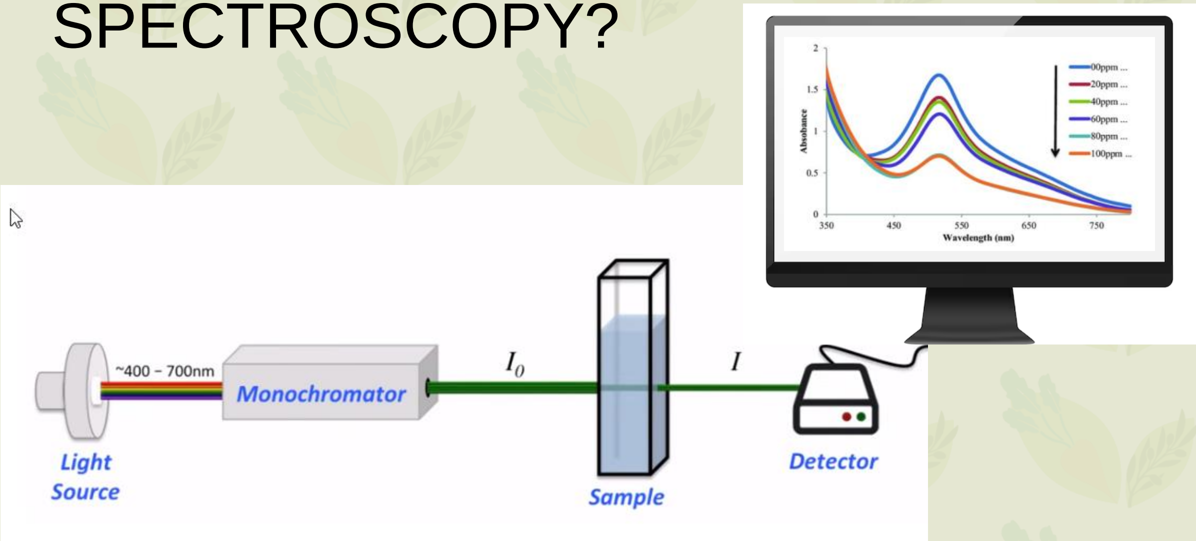
capture of ROS/RNS radicals, quantification

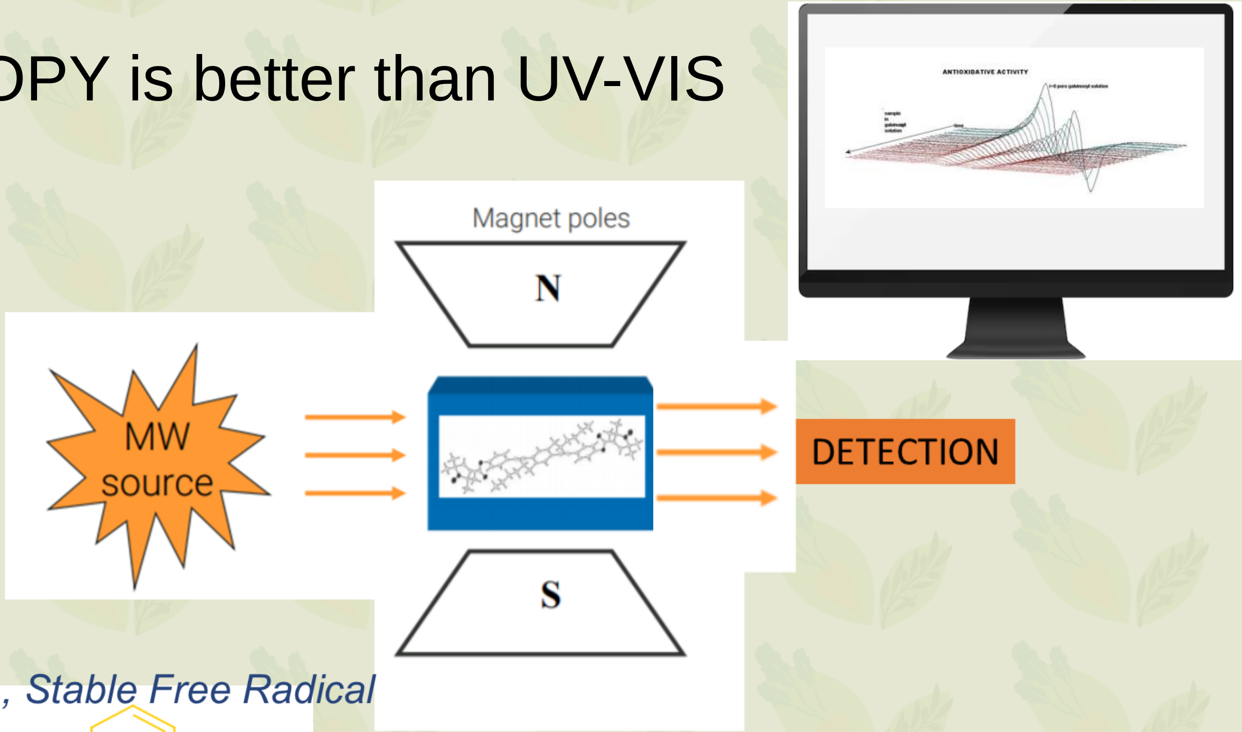
„searching for a needle in haystack”

IMPORTING/ADDITION OF RADICALS
FOR THE DETERMINATION OF ANTIOXIDANT CAPACITY



WHY we believe that EPR SPECTROSCOPY is better than UV-VIS SPECTROSCOPY?

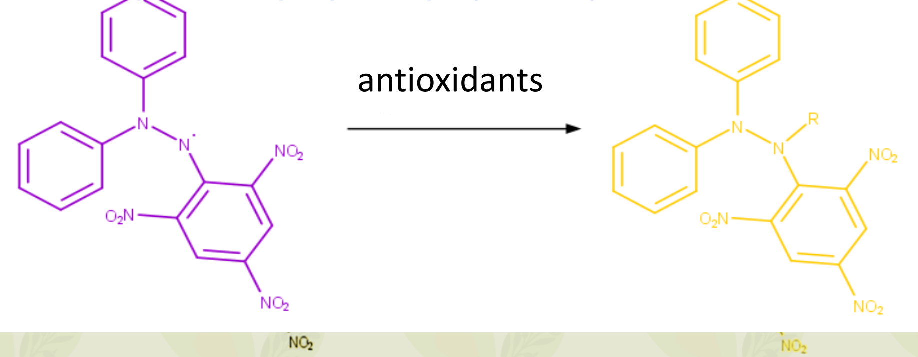




2 2-Diphenyl-1-picrylhydrazyl (DPPH), Stable Free Radical

REASON → **COLOR!!!**

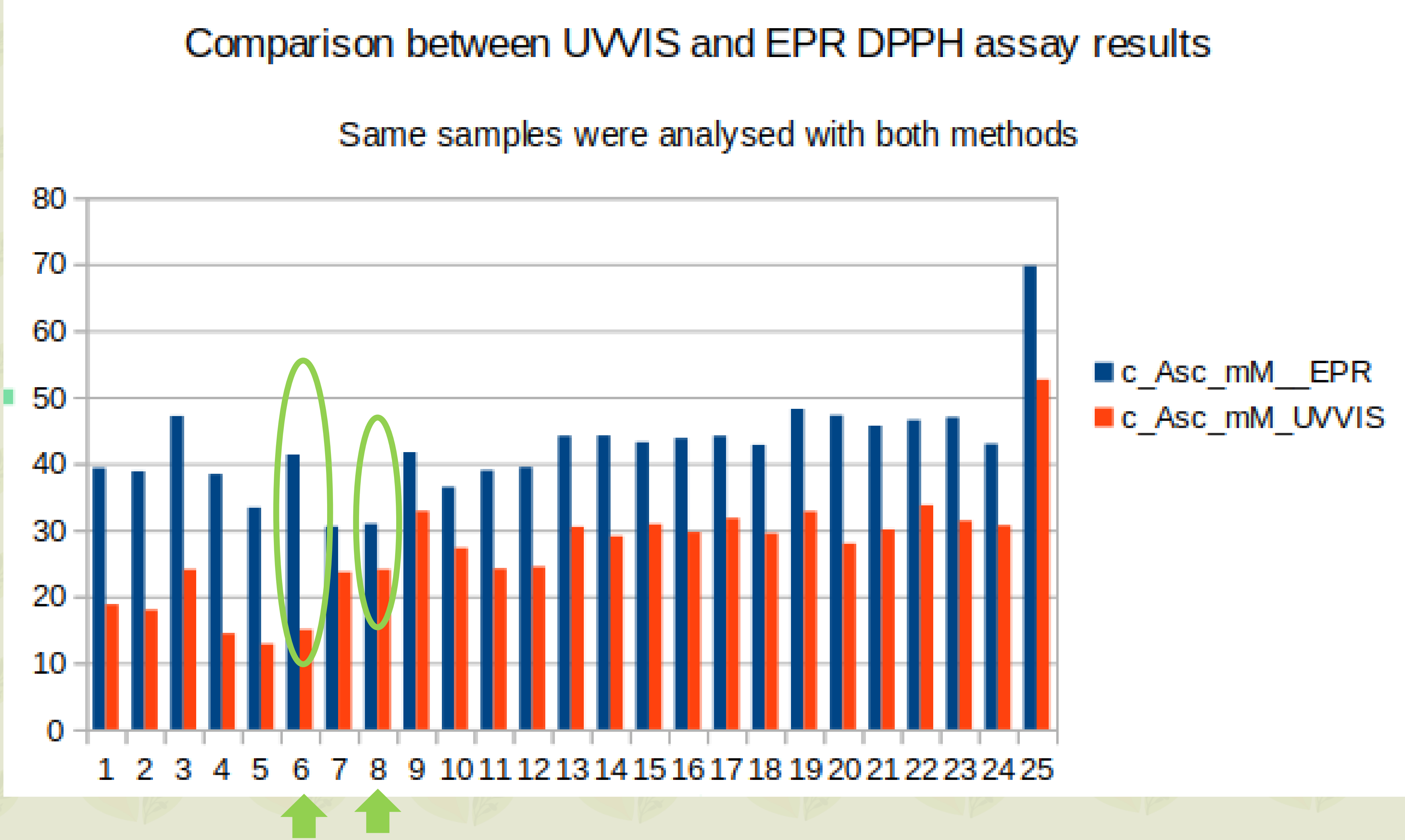
The reaction is not yet over with yellow color!



violet yellow

DPPH reacts with antioxidative species and transforms from a paramagnetic species (purple, EPR active) into a diamagnetic species (orange, EPR inactive)

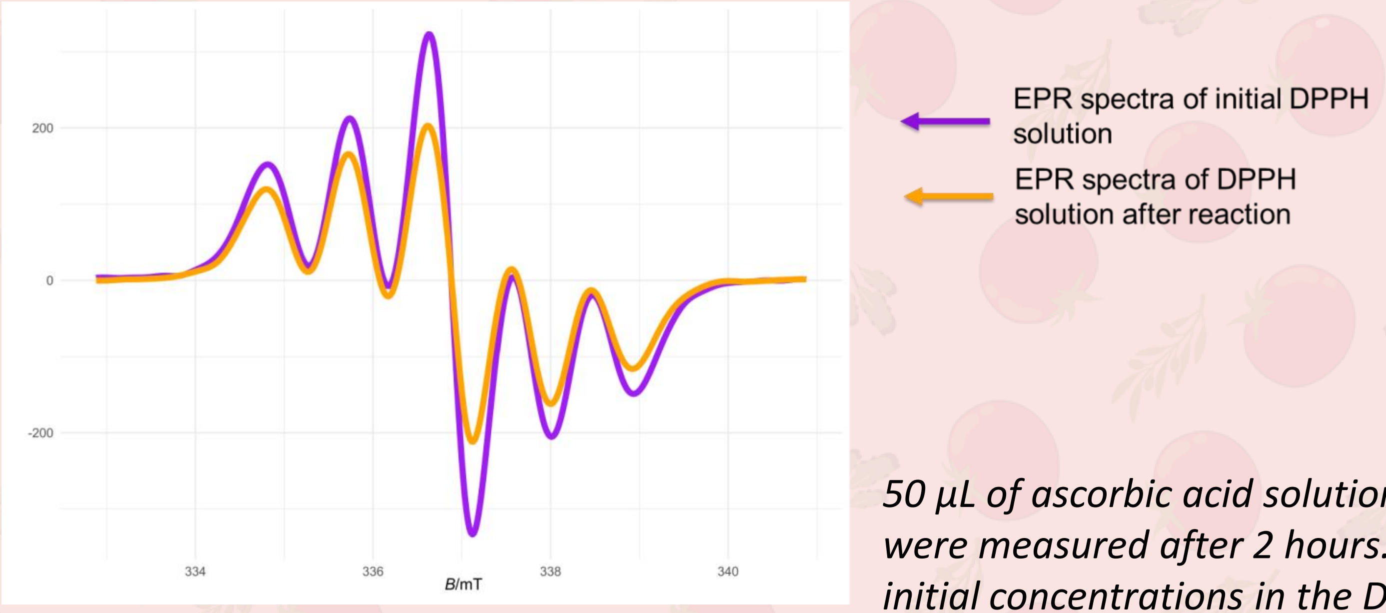
DISSADVANTAGES– SAMPLES WITH HIGH ANTIOXIDANT CAPACITY

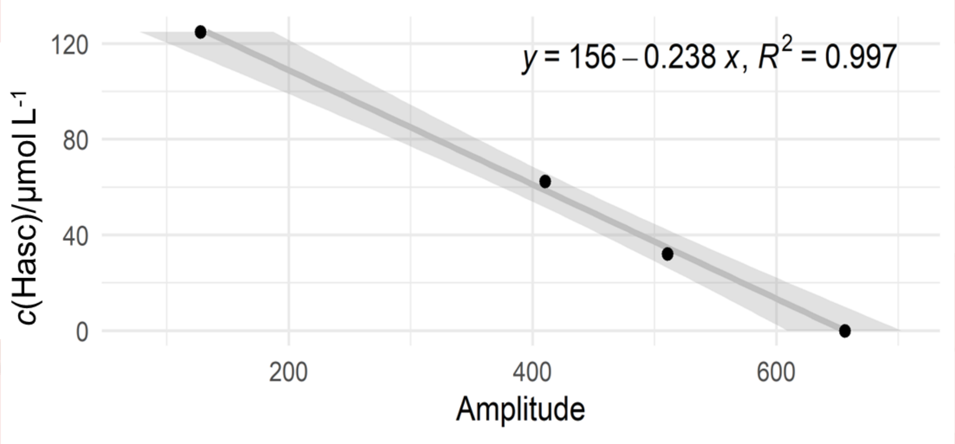


MODIFIED EPR PROTOCOL

The 1,1-diphenyl-2-picrilhydrazyl DPPH assay was used to determine all samples' free radical scavenging capacity. [1,2] Aliquots (50 μL) of the tested samples were mixed with 2.00 mL of a 0.3 mM ethanolic solution of the DPPH radical and a blank sample consisting of 50 μL of water.

After two hours, the concentration of DPPH radical was determined by EPR spectroscopy.

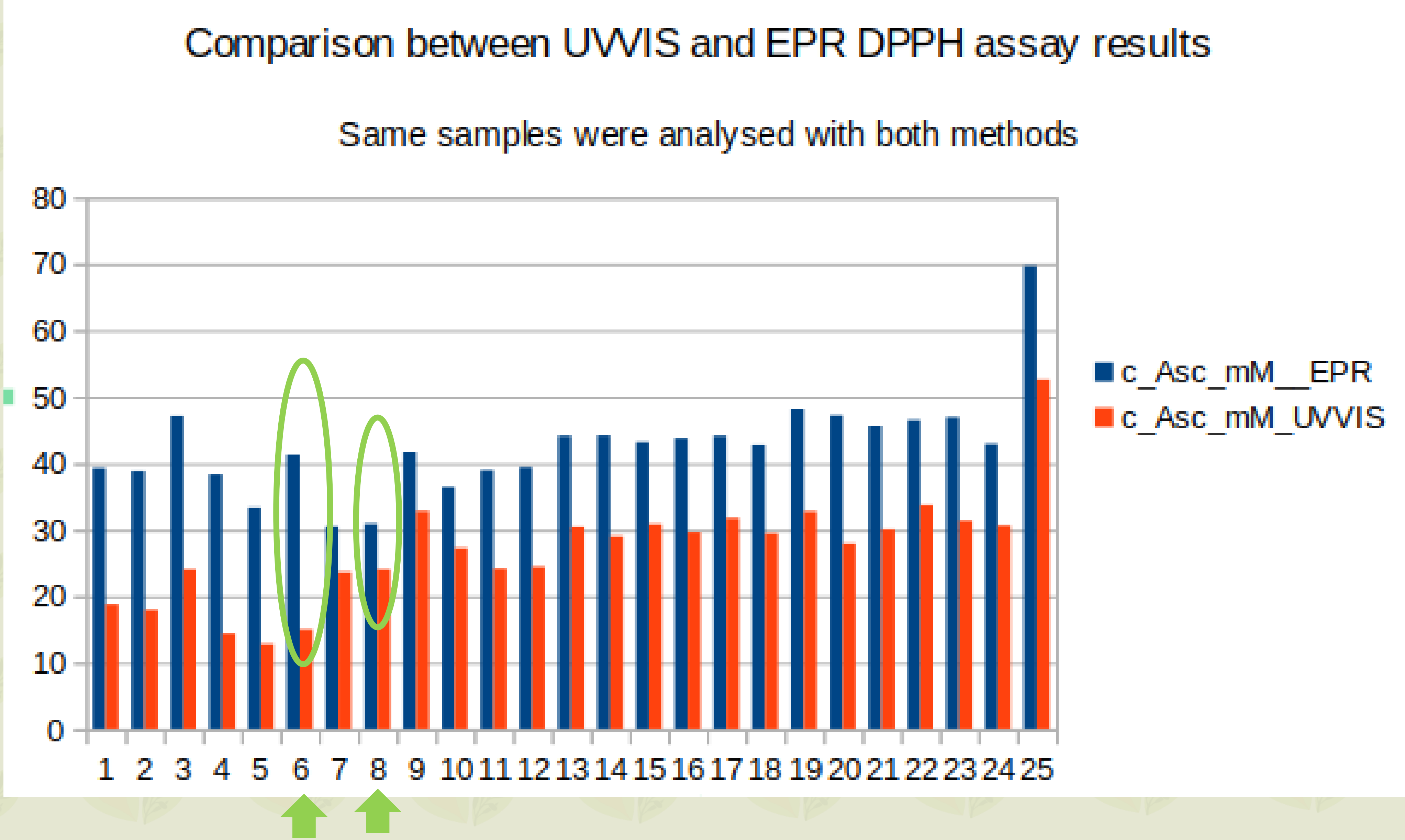




50 μL of ascorbic acid solution was added to 2.0 mL of the DPPH solution. The EPR spectra were measured after 2 hours. The concentrations of the solutions used (c_o(HAsc))) and the initial concentrations in the DPPH mixture(c(HAsc))), which were used to generate the calibration curve.

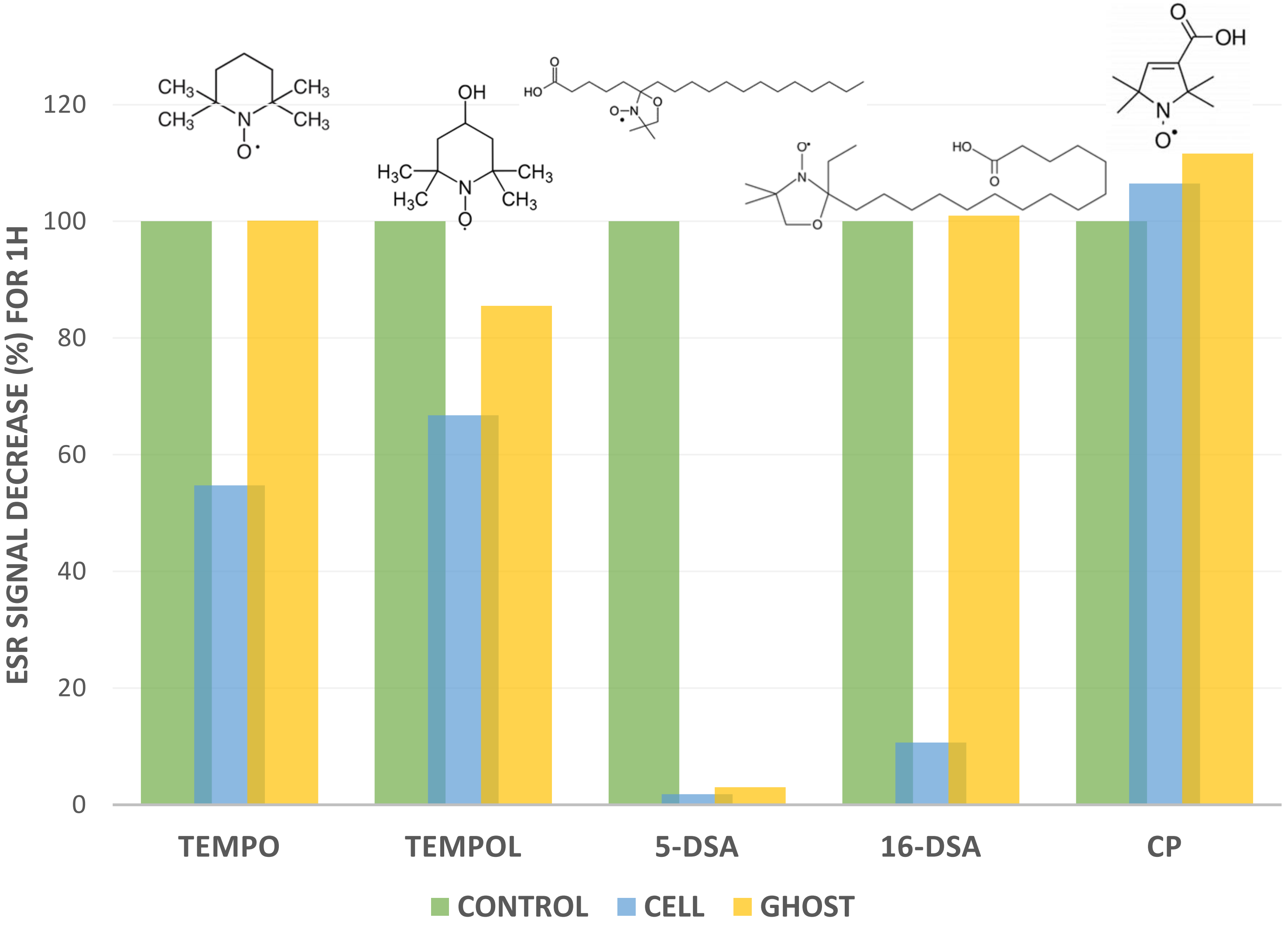
1. Fuhrman, B., Volkova, N., Suraski, A., Aviram, M. (2001) J. Agric. Food Chem. 49(7), 3164-8.
2. Von Gadow, A., Joubert, E., Hansmann, C.F. (1997) Food Chem. 60, 73-77.

DISSADVANTAGES– SAMPLES WITH HIGH ANTIOXIDANT CAPACITY



ANTIOXIDANT ACTIVITY OBTAINED BY EPR/ESR SPECTROSCOPY

Microalgae vs Microalgae under oxidative stress (ghost)



- difference in the reducing agent in cell and ghost (amount of chlorophyll, carotenoids, etc.)
- nitroxides are a group of compounds highly suitable for protection against oxidant stress
- relationship between nitroxide structure (spin probe) and nitroxide reduction rate (antioxidant activity)
- charged reducing agents favor opposite charged or uncharged nitroxides and therefore facilitate the electron exchange between the two molecules (chlorophyll molecule a positive charge)

DISSADVANTAGES– SAMPLES WITH HIGH ANTIOXIDANT CAPACITY

Microalgae
Dunaliella tertiolecta CCMP 1320

SOURCE
Culture Collection Bigelow
Laboratory for Ocean Sciences,
Bigelow, MN, USA

Lyophilized powder

&

Sample	Microalgae *Dunaliella tertiolecta* % w/w	c(ascorbic acid eq.)/mmolL^{-1}	
Algae in filtered seawater	1.0	25 ± 2	45.3 %
Algae in citrate buffer (pH4.4)	1.0	26 ± 2	47.0 %
Tomato juice	0	20 ± 2	37.8 %
Algae in tomato juice	0.5	25 ± 2	45.7 %
	1.0	23 ± 2	42.7 %
	1.5	25 ± 2	46.6 %

CONCLUSION
The addition of freeze-dried microalgae *Dunaliella tertiolecta* improves significantly the antioxidant properties of tomato juice even in small amounts (0.5-1.5% w/w).

20-23% considerable increase
of antioxidative capacity

UWave-2000 Multifunctional Workstation

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Ultrasound tretament

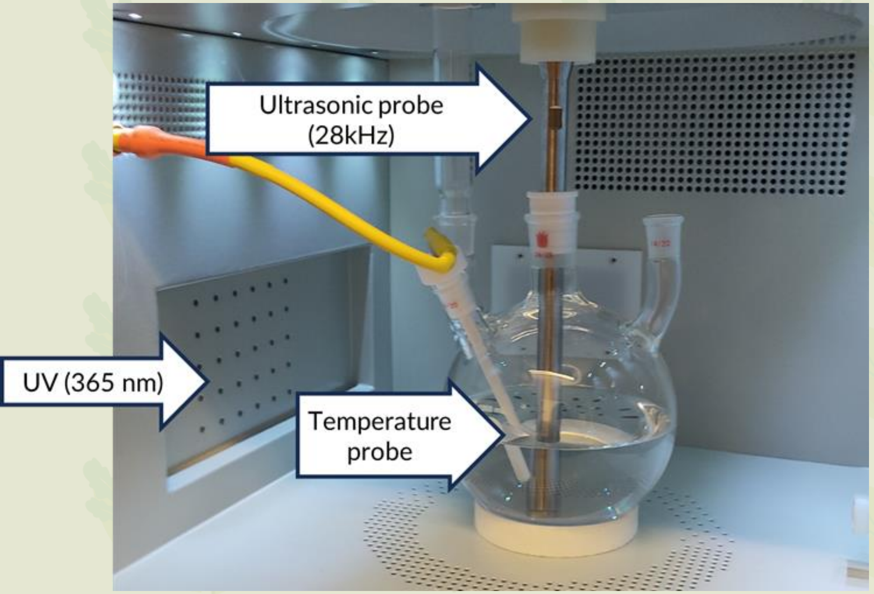
- Continuous
- Pulse
  (time, power, probe type amplitude, ΔT, E)

Microbiological research

- Standard total plate count (TPC)
- Total mold counts (TMC)
- E. Coli
- Bacillus coagulans confirmation method

Physical Chemistry Characterization

- Dry matter/Humidity
- Brix
- pH
- Conductivity
- Total Phenolic and Antioxsidant activity (UV-Vis and EPR)
- Color
- Total Acidity (TA)
- Lycopene; B-carotene; chlorophyll B; chlorophyll A content



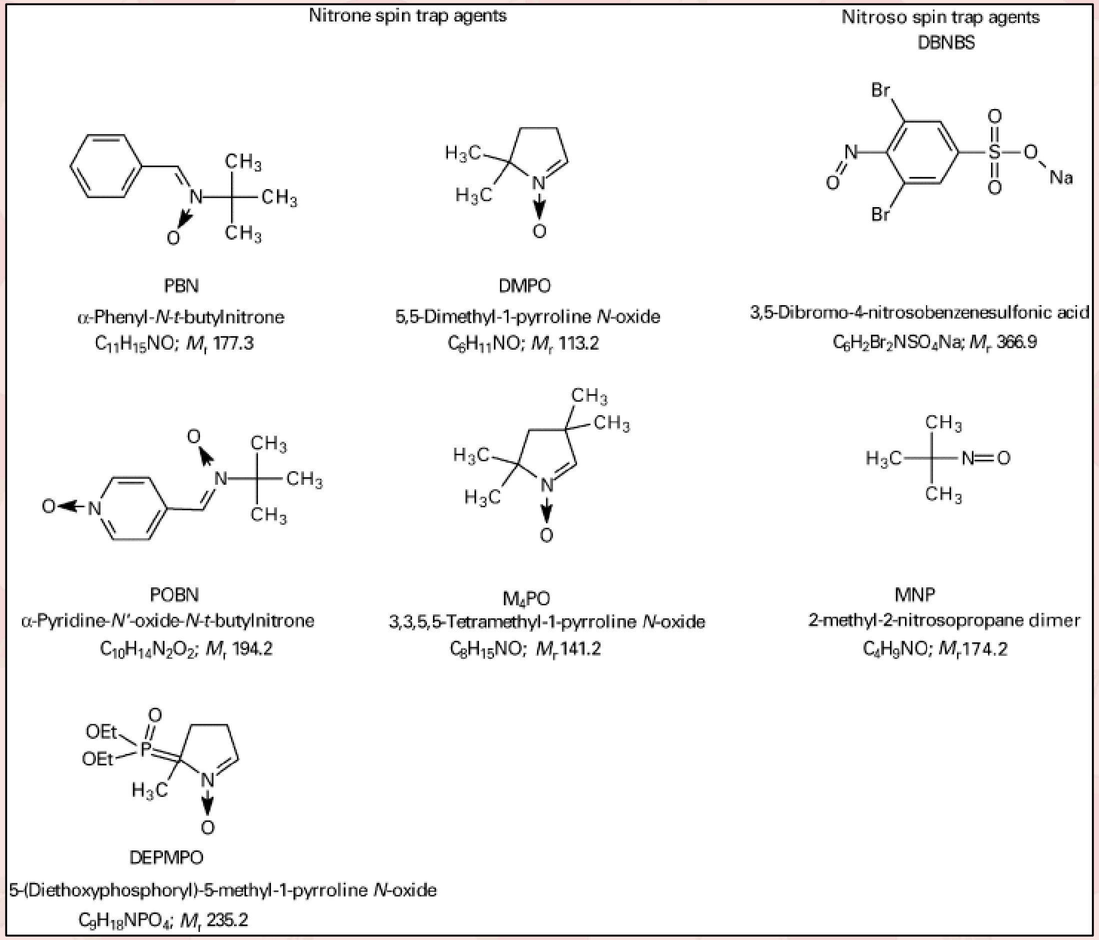
Experimental procedure:

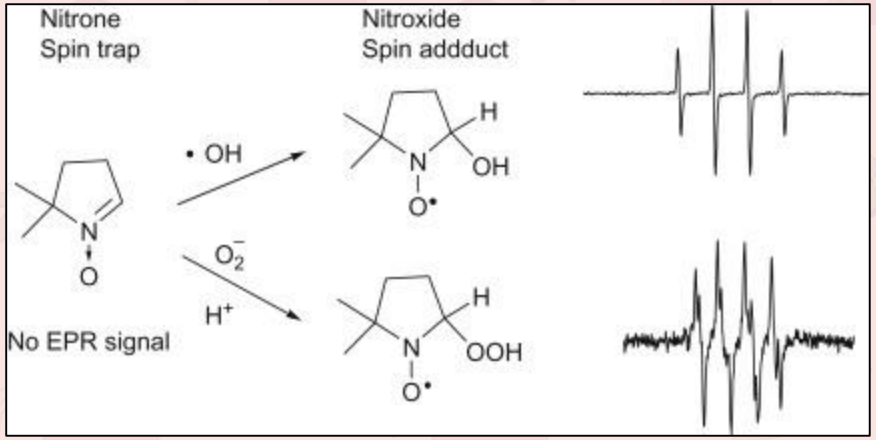
- Mechanical homogenization, 3 min,10000 rpm
- Simultaneous UV/US treatment in the Uwave 2000 microwave reactor
- Characterization after each consecutive treatment

ROS/RNS SPIN TRAPING

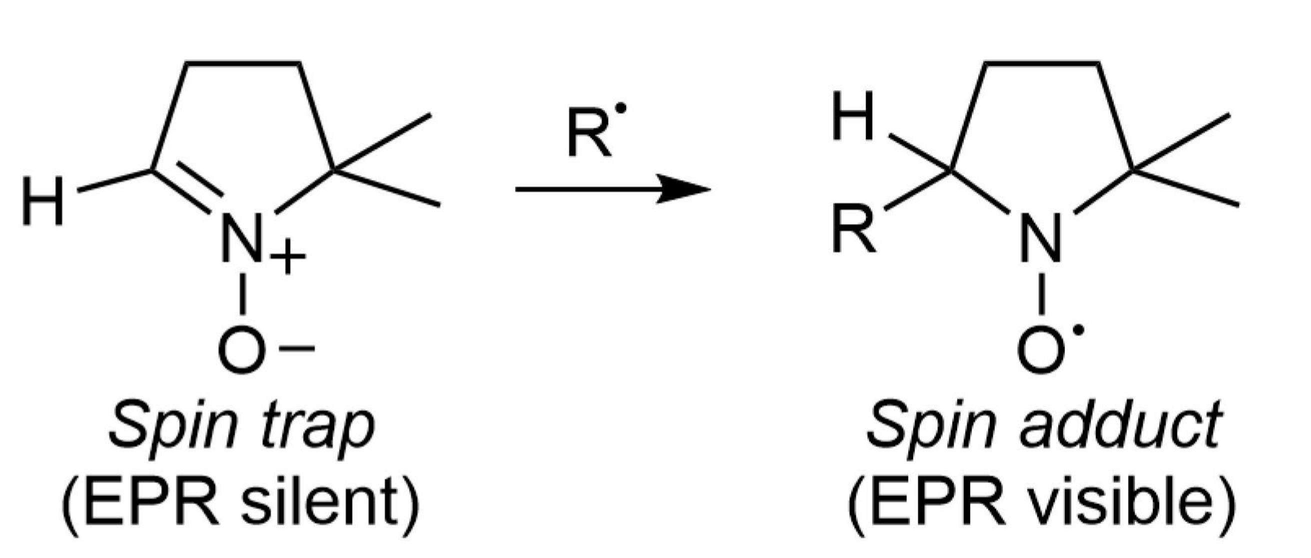
The life-time of organic free radicals is usually very short because they undergo bimolecular self-reaction.

Spin traps are diamagnetic molecules exerting a particular high affinity for reactive radicals, to which reactive radicals rapidly add to form persistent spin adducts, detectable in the EPR spectroscopy.

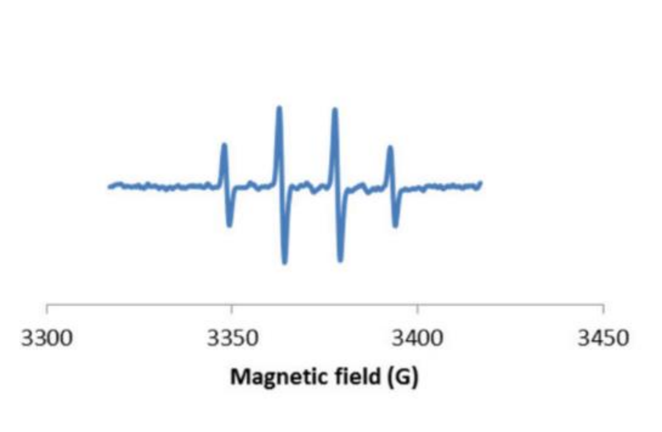




DMPO - the most widely used EPR spin trapping agent



The principle of spin trapping with DMPO. The short-lived radical R• adds to the cyclic nitrone and forms a stable nitroxide radical that can be detected by EPR.



Positive control spectra

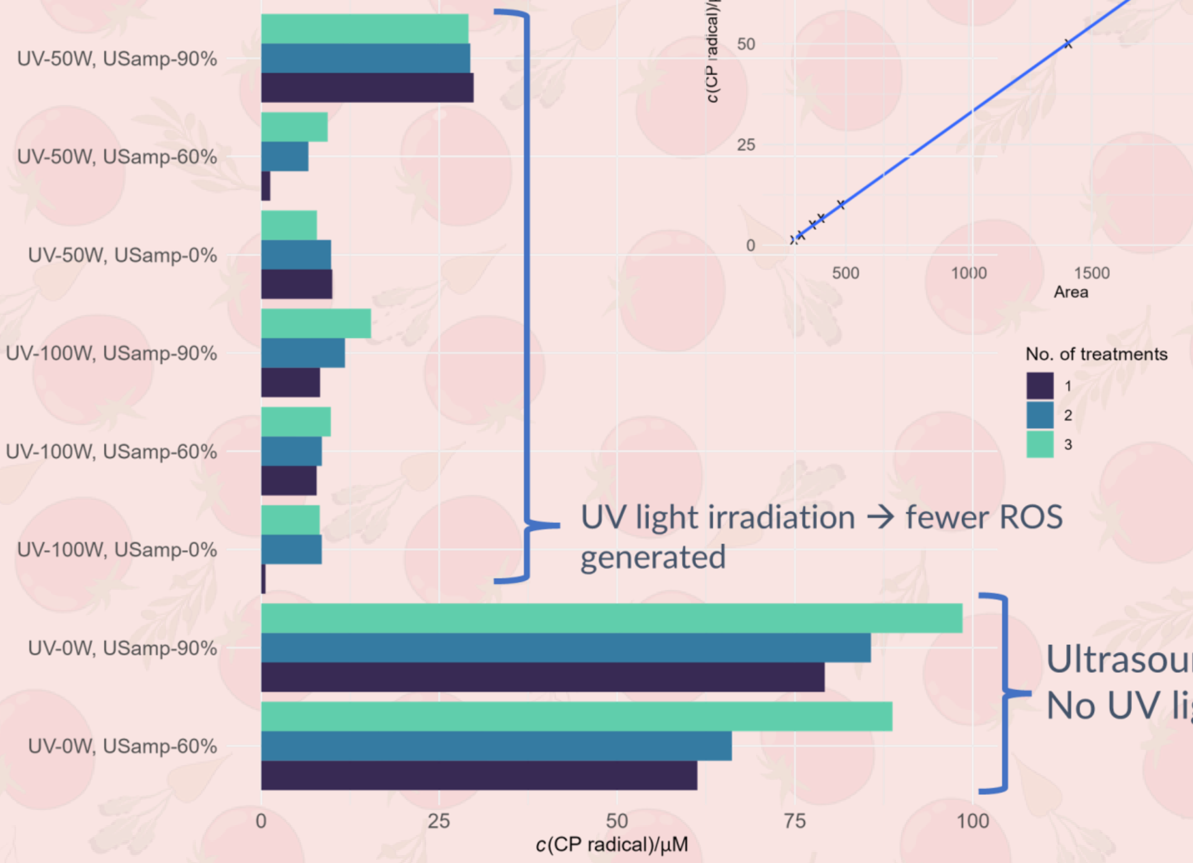
Tomato samples – no EPR signal!

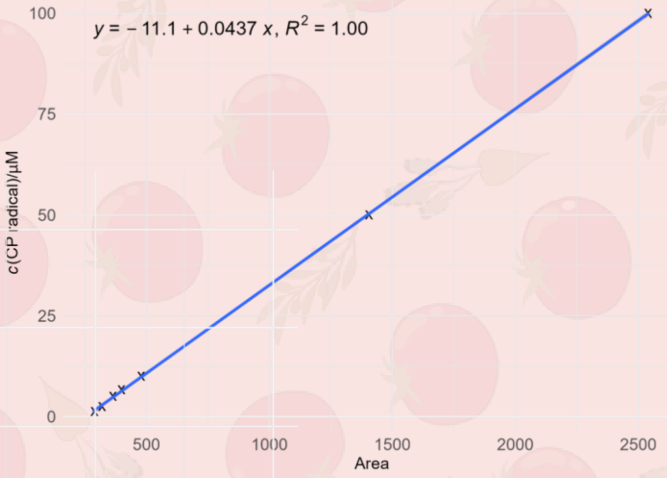
POSSIBLE REASONS

-the treatment (UV and/or US) reduces the concentration of the spin trap

-relationship between the nitroxide structure (spin trap) and the nitroxide reduction rate (antioxidant activity)

The area under the EPR signal is proportional to the concentration of the CP˙ radical

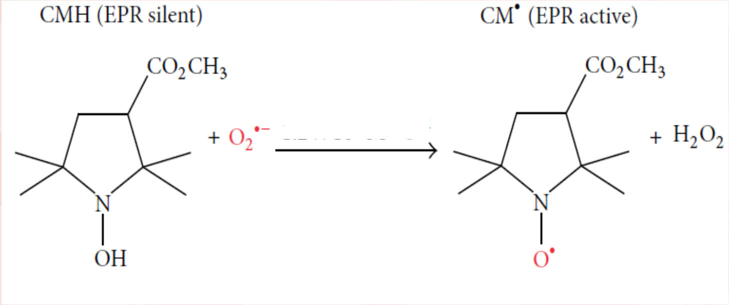




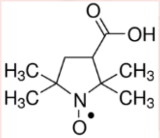
UV light irradiation → fewer ROS generated

Ultrasound treatment
No UV light irradiation

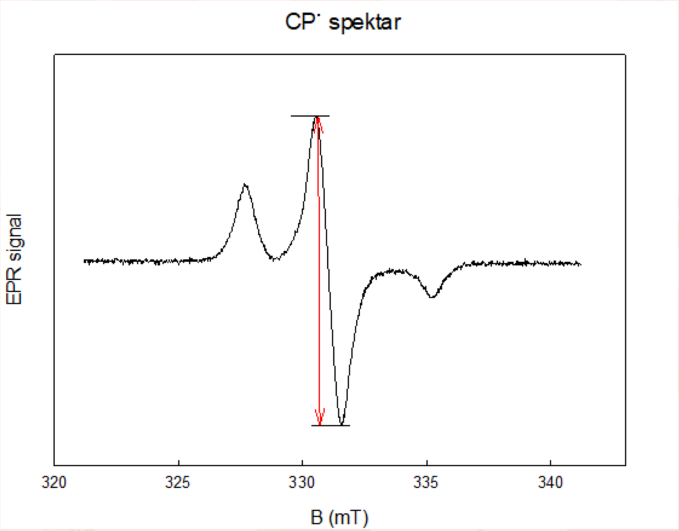
1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH)



superoxide radical concentration detected



3-carboxy-proxyl (CP•)



NEXT STEPS

- New synthesis of the EPR spin trap
- Experiments with different treatments (HP, UV, MW, US, IR, IC and combinations)
- Optimization of EPR protocols

POSTER PRESENTATION

P1.2.128

Thank you for your attention!

RBI & FER TEAM:

Nadica Maltar Strmečki
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