SENSIBILIZED BIOSENSORS FOR RADICAL AND ANTIOXIDANT PROPERTY DETERMINATION

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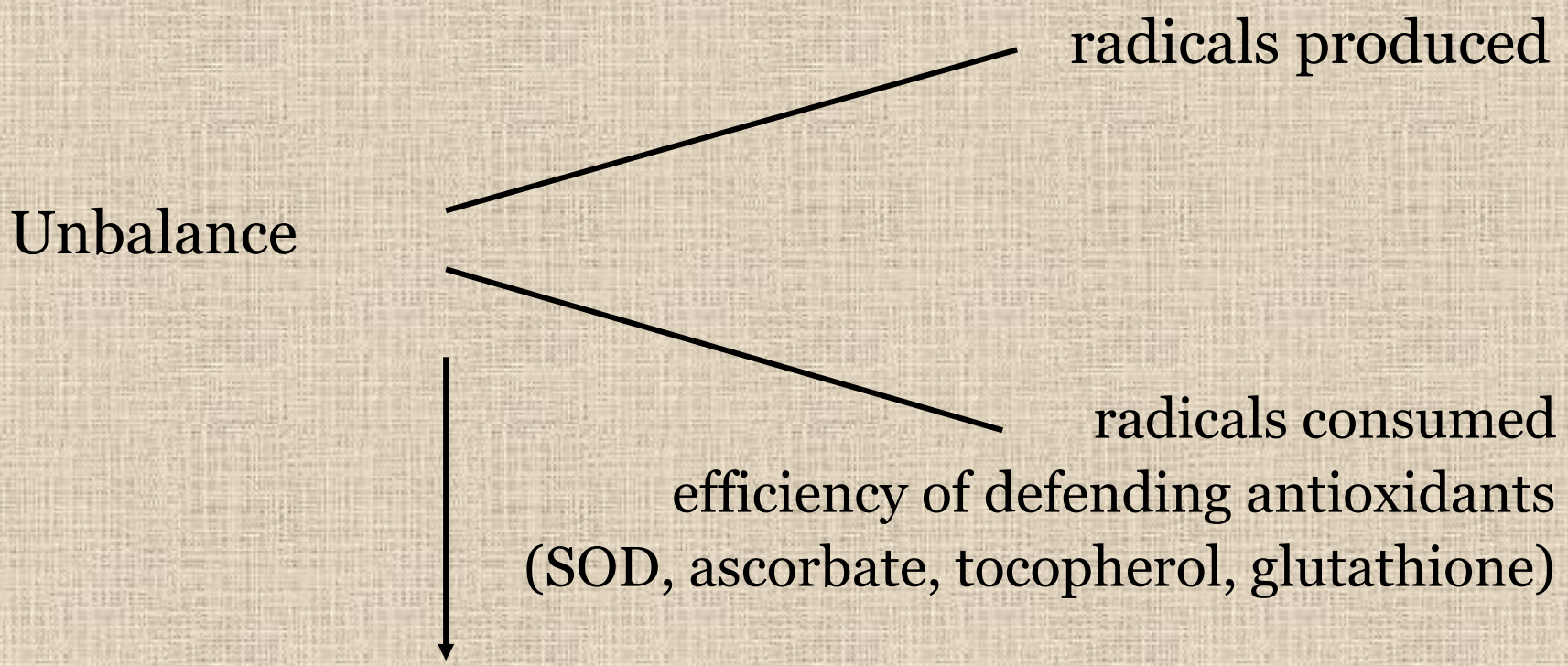
RADICALS OF OXYGEN

- Oxidation numbers of Oxygen
  - 0 (molecular oxygen)
  - -0.5 superoxide free radical
  - -1 (hydrogen peroxide)
  - -1.5 hydroxide free radical
  - -2 (water)

Oxidative

Stress



Determination of radicals

ESR: Electron Spin Resinance

Colorimetric tests (reaction of nitrotetrazolium blue chloride; nitrocathecol generated by nitrophenol)

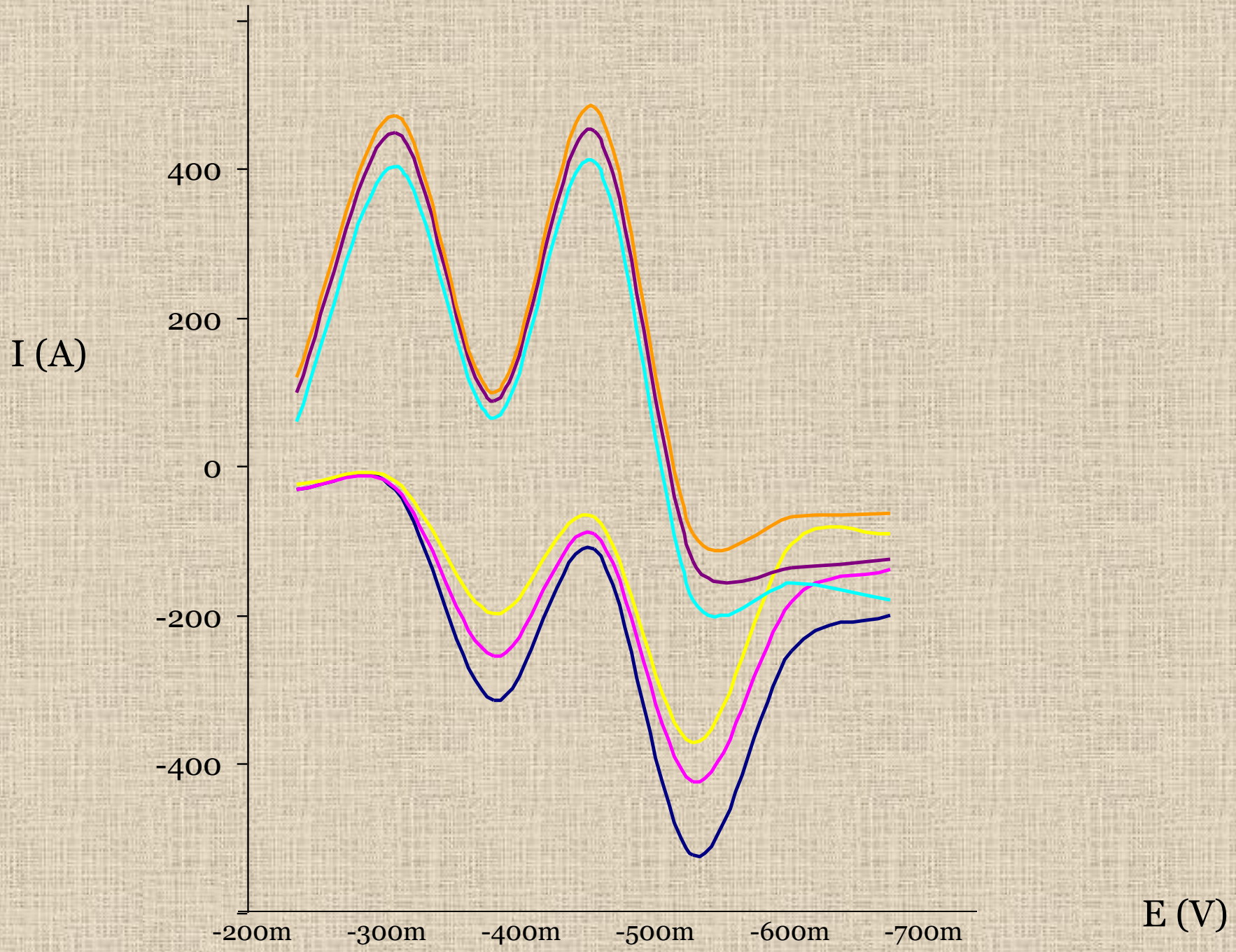
Fluorimetry

RADICALS → DETERMINATION BY CHEMICAL METHODS

BIOSENSOR BASED ON SUPEROXIDE DISMUTASE ENZYME

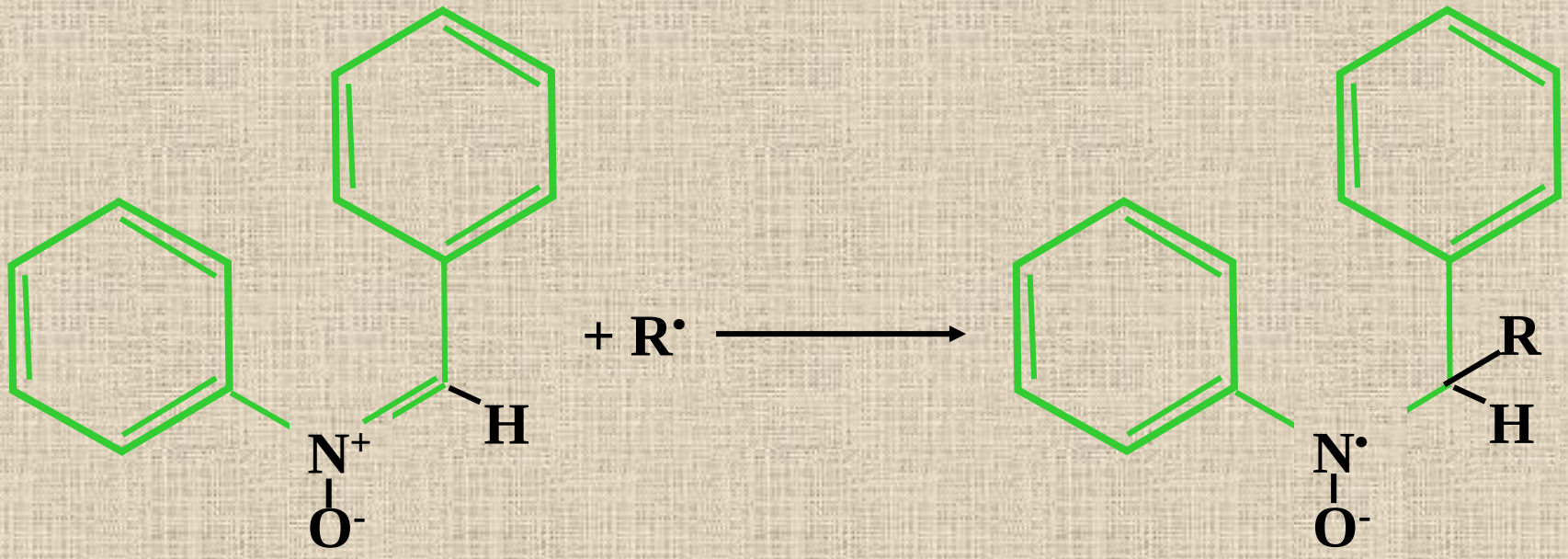
DISMUTATION CAPACITY

CYCLIC VOLTAMMETRY BASED ON THE MEASUREMENT OF THE ANODIC AREA


I (A)
400
200
0
-200
-400
-200m
-300m
-400m
-500m
-600m
-700m
E (V)

The principle of the method is as it follows; antioxidant capacity is strictly related to reduced form concentration of natural products; these forms contribute to the anodic run of a cyclic voltammetric curve; the area subtended by the anodic profile can be assumed as proportional to the integral antioxidant capacity. By this way some common foods as: coffee, tea, tomato, radish, cabbage, and some drugs or natural compounds as vitamins C and E, butyrrylhydroxyanisole and pyrogallate were studied obtaining results in good agreement with literature data and enzymatic results.

Potentiometric determination of free radicals using a benzylidenphenylnitrone modified electrode



Benzylidenphenilnitrone radical adduct

Variation of membrane potential; the same by FET system

Amperometric determination of superoxide radicals using a cytochrome modified carbon paste electrode

$$O^{\bullet}_2 + \text{cytochrome } c^{3+} \longrightarrow O_2 + \text{cytocrome } c^{2+}$$

$$\text{cytochrome } c^{2+} + [\text{Fe(III) - protoporphyrin IX}] \longrightarrow \text{cytocrome } c^{3+} + [\text{Fe(II) - protoporphyrin IX}]$$

$$[\text{Fe(II) - protoporphyrin IX}] \longrightarrow [\text{Fe(III) - protoporphyrin IX}]$$

Measurements of oxidative current of reduced cytochrome

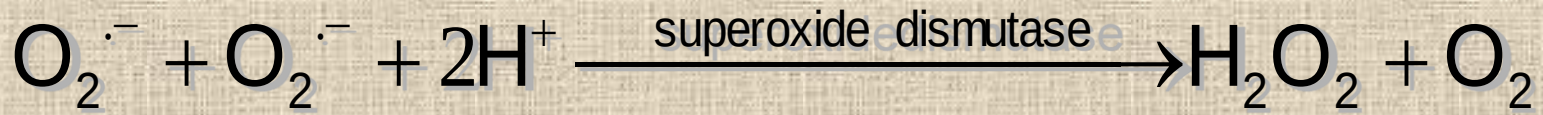
Superoxide dismutase and oxidation protective enzymes

$$O_2^- + O_2^- + 2H^+ \xrightarrow{\textit{superoxidase dismutase}} H_2O_2 + O_2$$

$$H_2O_2 + H_2O_2 \xrightarrow{\textit{catalase}} 2H_2O + O_2$$

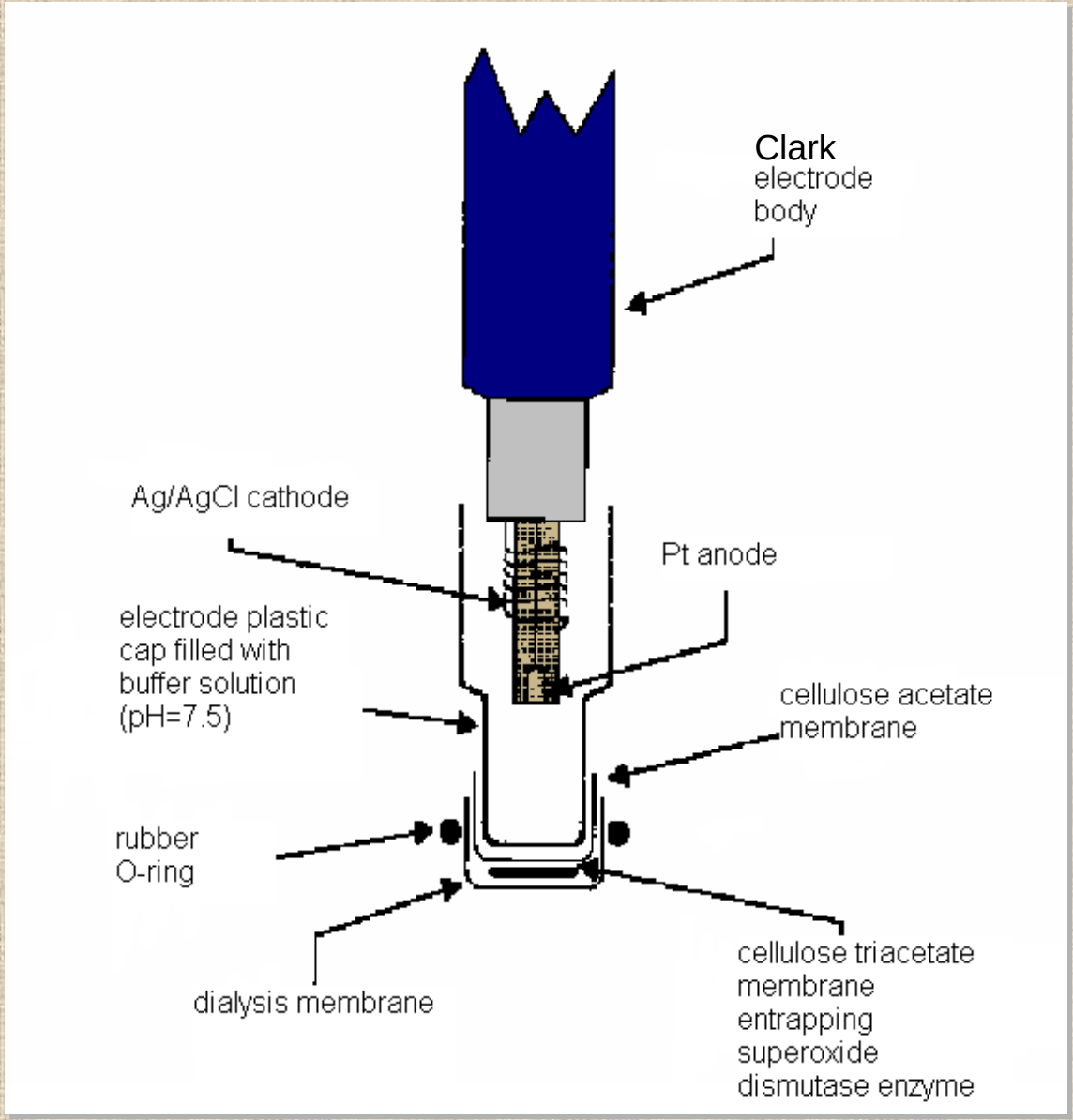
$$H_2O_2 + H_2A \xrightarrow{\textit{peroxidase}} 2H_2O + A$$

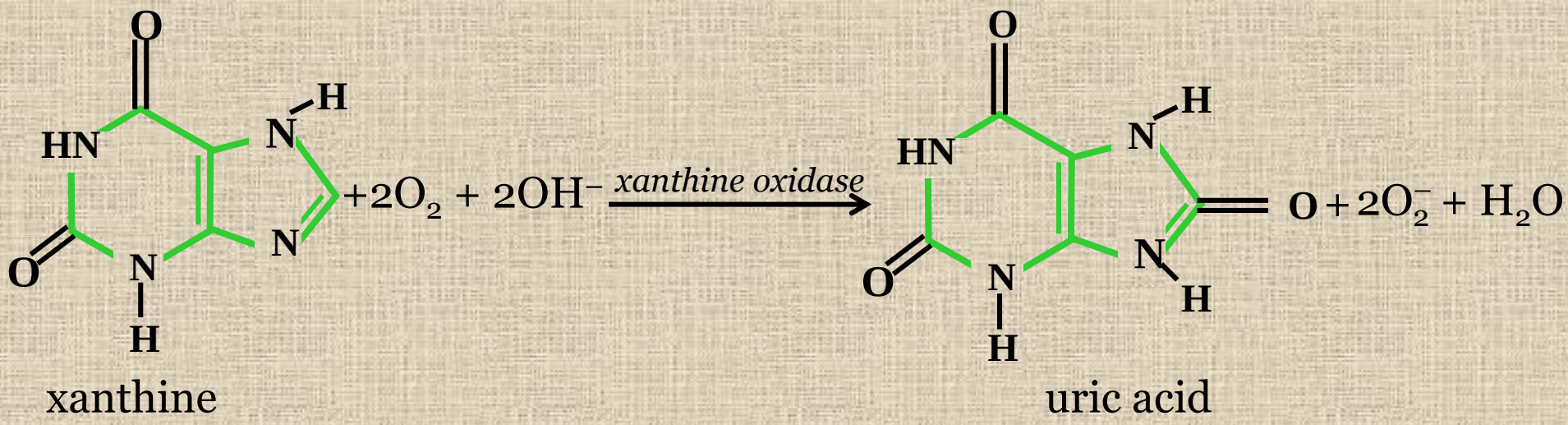
The biosensor is based on superoxide dismutase enzyme and on the following reaction



that is the dismutation of superoxide into oxygen and hydrogen peroxide anodically monitored by an amperometric Clark sensor acting as trasducer

Superoxide dismutase biosensor assembly

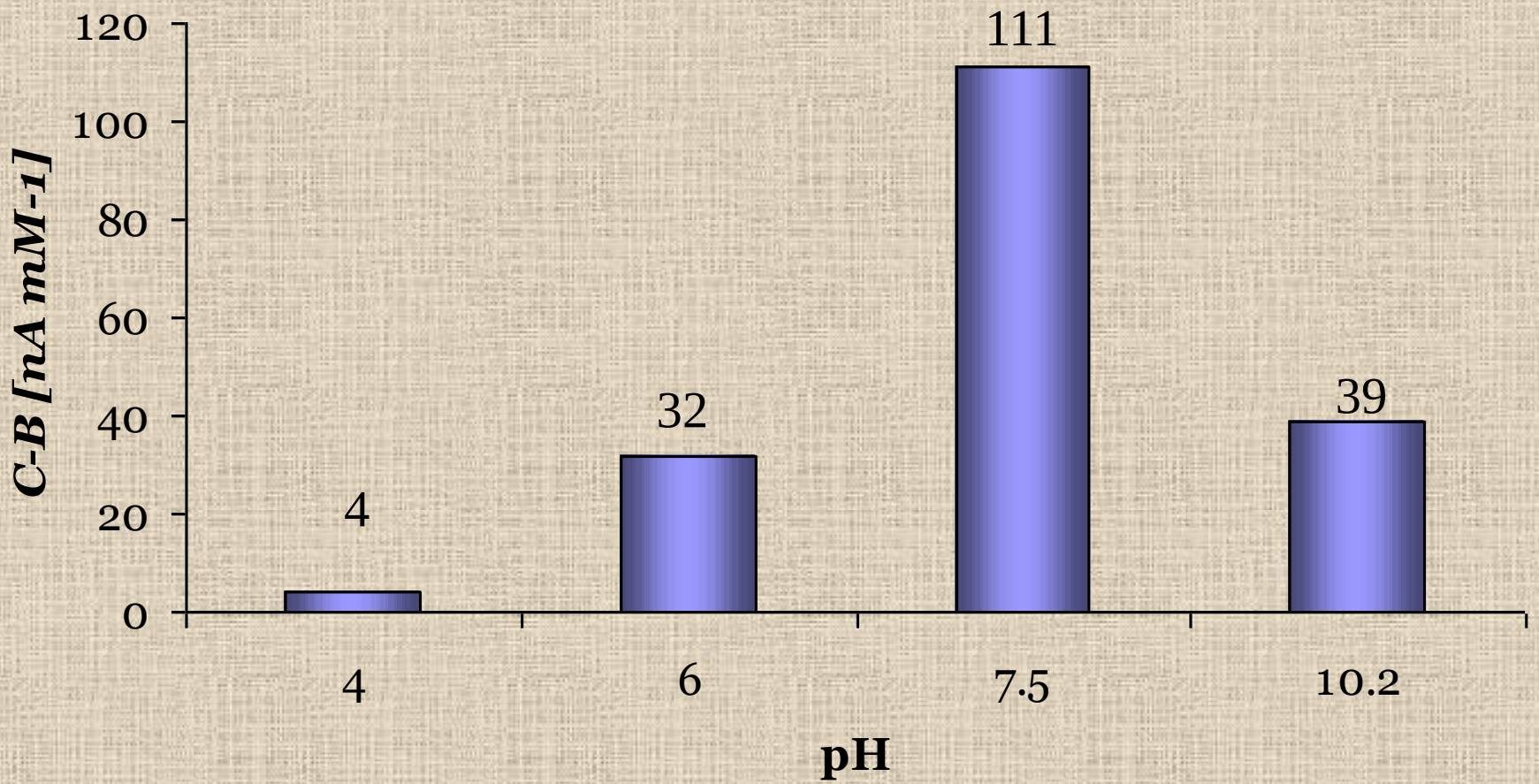




xanthine

uric acid

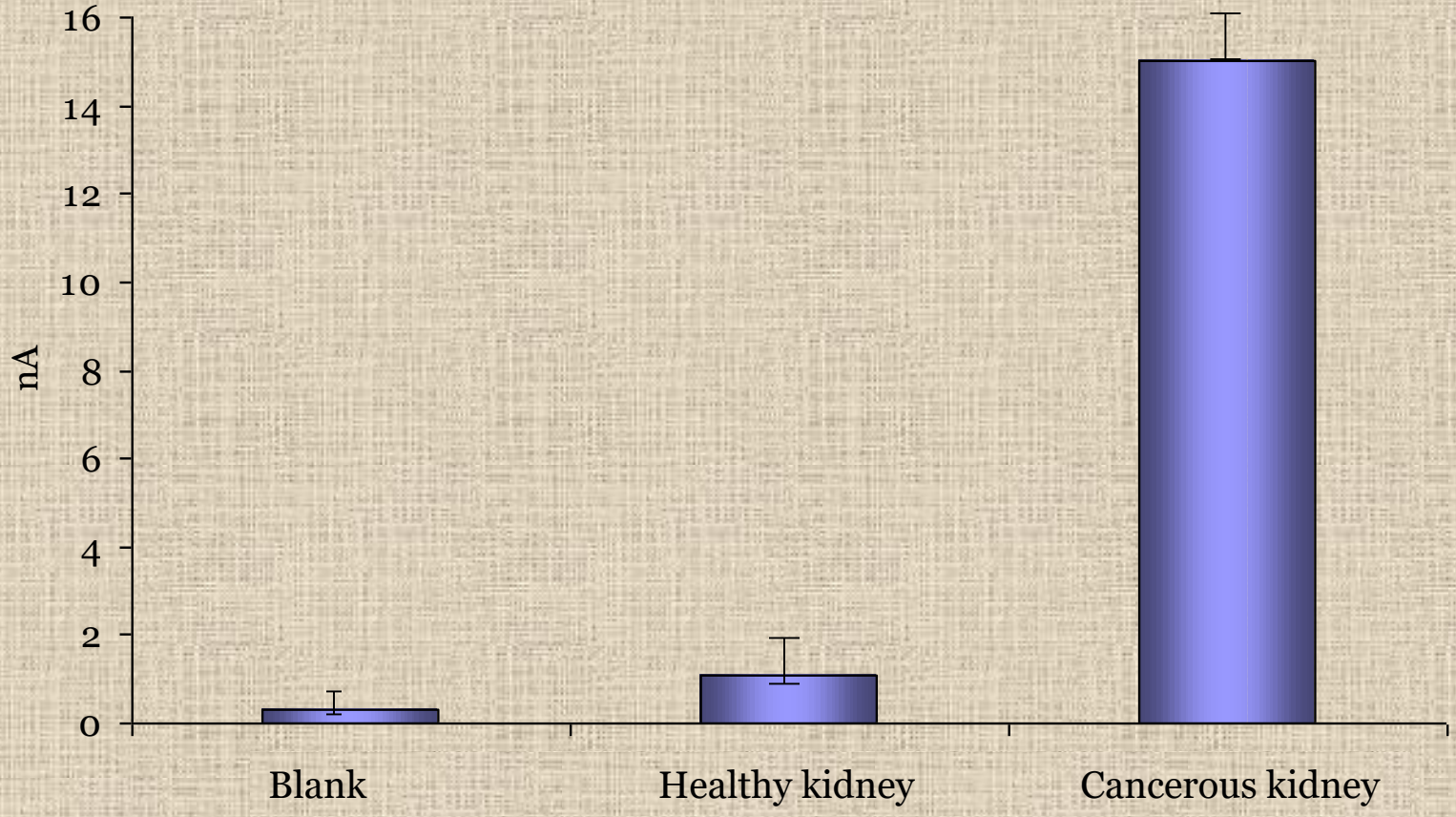
Generation of superoxide radicals for calibration



Trend of the sensitivity of the SOD/H_2O_2 biosensor (as difference of biosensor's and "blank's" slope values at different pH) working in different aqueous buffer solutions; pH=4 acetate buffer, pH=6 phosphate buffer, pH=7.5 phosphate buffer, pH=10.2 carbonate buffer

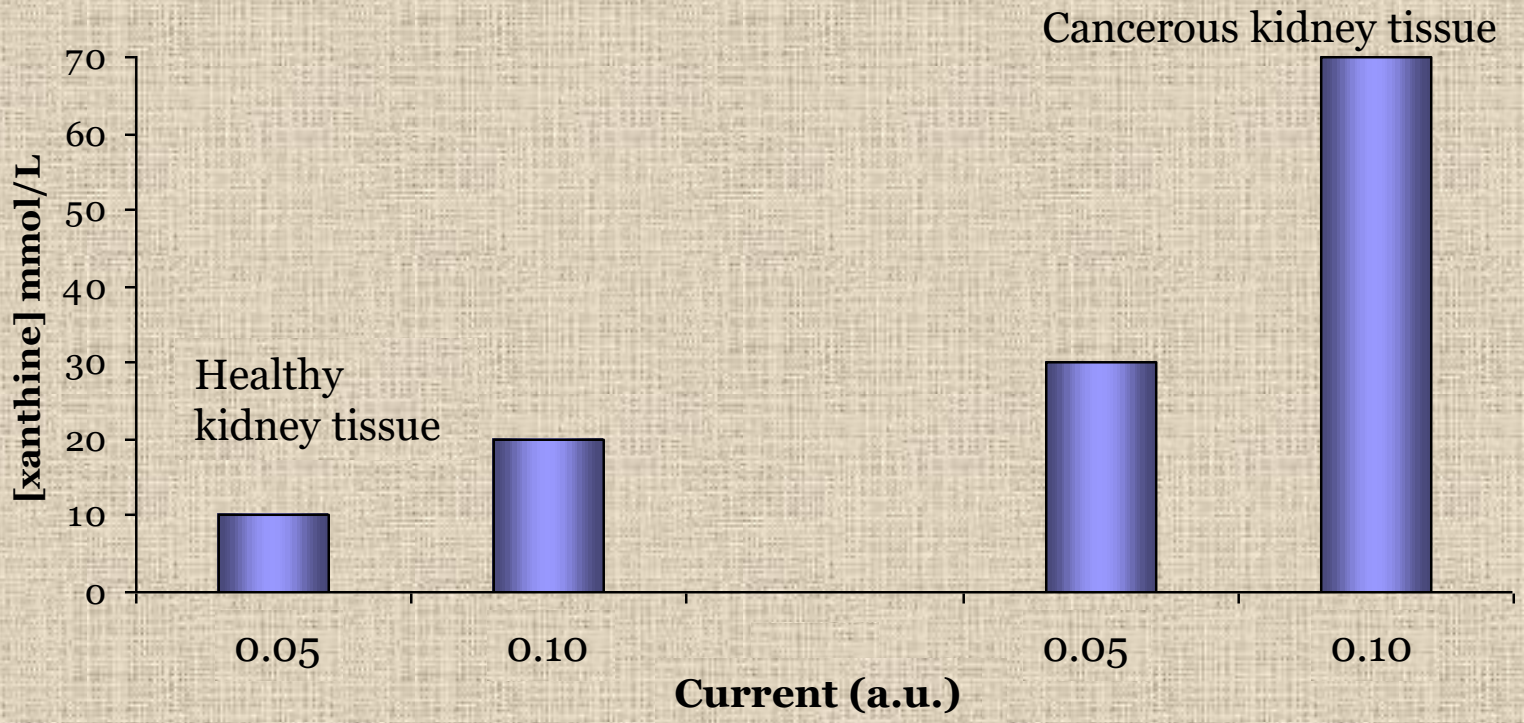
Tests on healthy and sick tissues

The following protocol was used for the tests: the biosensors was allowed to stabilize in 20 ml of phosphate buffer solution 0.01 M, pH=7, under magnetic stirring, until the response was constant; at this stage a solution containing homogenised healthy or cancerous kidney tissue (0.5 g of tissue in 3 ml of distilled water) was added and the biosensor response, that is the current variation that occurred after adding the homogenised tissue, was then recorded. Before the use the tissue was stored at -20°C.



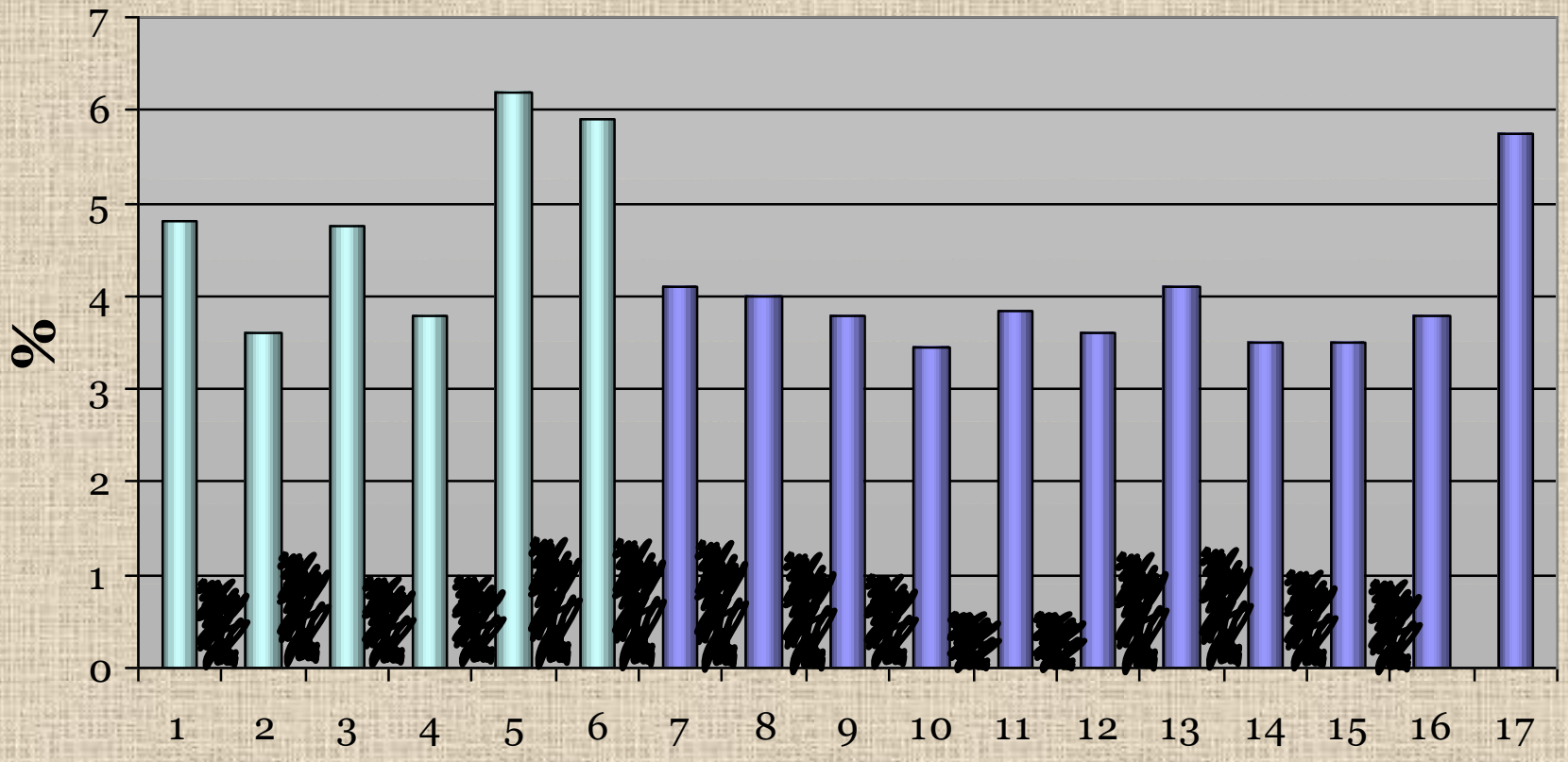
Signal variation of the superoxide dismutase biosensor after addition of homogenised healthy and cancerous kidney tissue aqueous extracts. Each value is the mean of at last three determination

Signal variation, in presence of xanthine oxidase, after addition of the same two different concentrations of xanthine to aqueous extracts of healthy and cancerous kidney tissue



Metabolic capacity (optimum for 1 mg/ml proteic concentration) determined by carbon 14 marked benzene incubated with kidney tissue samples.
Produced muconic acid determined by HPLC

% metabolized benzene by kidney tissue



Three series: 1 (white) and 2 (grey) healthy, 3 (black) cancerous tissue

Higher concentration of free radicals

1. Lowered superoxide dismutase activity.

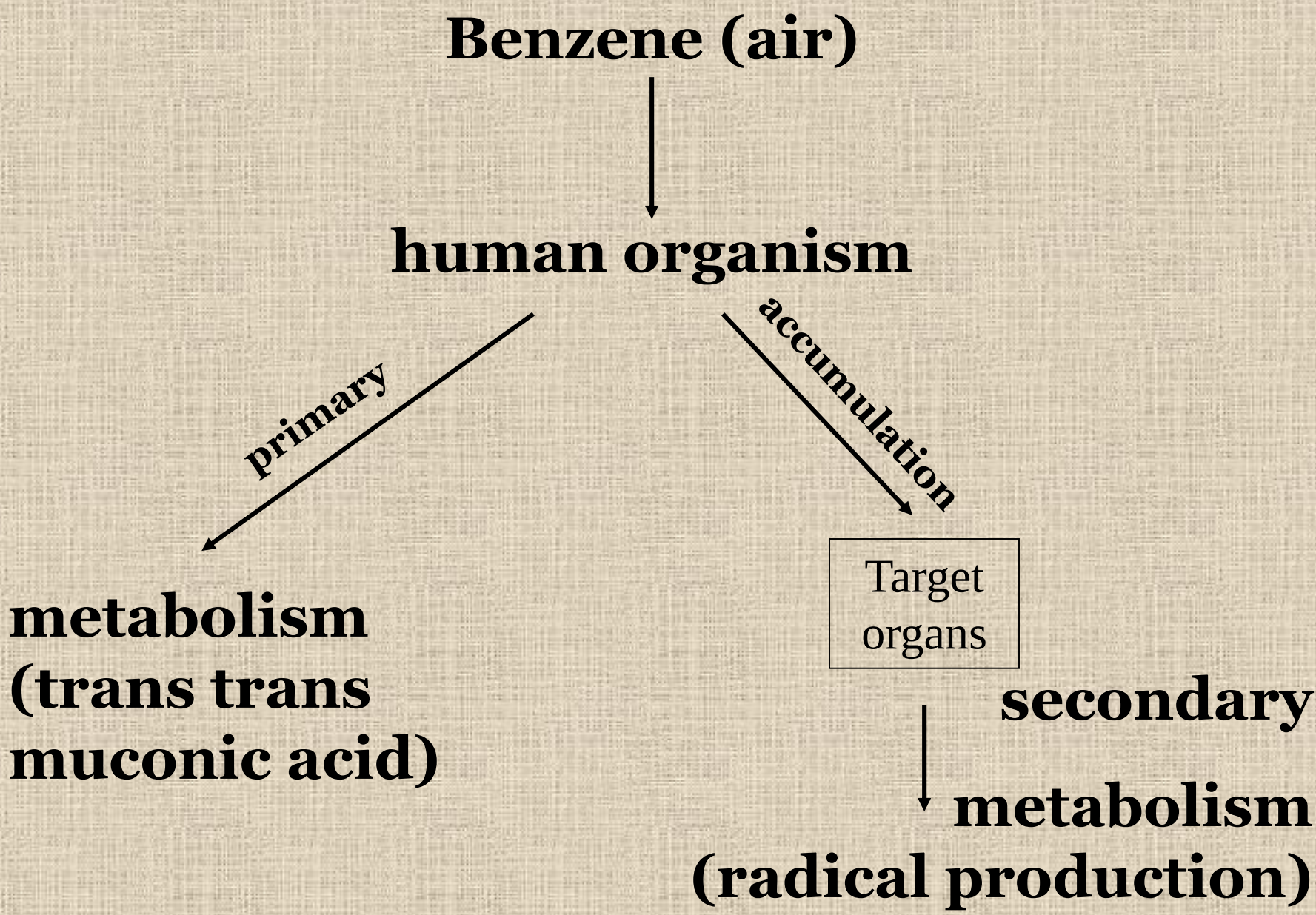
2. Production of free radicals by secondary pathways of benzene metabolism due to less active dioxygenase.

3. Lowered free cysteine (natural radical scavenger) concentration due to a Lewis acid base reaction between heavy metal ions and methallothionins.

$pK_{Carb} = 1.71$ $pK_{Amm} = 10.77$

$pK \cong 8$ (complex)

stability constant with heavy metals


Benzene (air)
human organism
primary
accumulation
metabolism
(trans trans
muconic acid)
Target
organs
secondary
metabolism
(radical production)

Examined tissues (benzene conc µg/g)	
kidney	
liver	**1,59 ± 0,05**
intestine	**0,39 ± 0,02**
testicle	**2,44 ± 0,05**
lung	**0,49 ± 0,02**
muscle	**4,25 ± 0,08**
fat	**2,52 ± 0,06**

Kidney

healthy patient 0,99 µg/g ± 0,03
suffering from cancer patients or deads

1,43 ± 0,05
(healthy tissue)

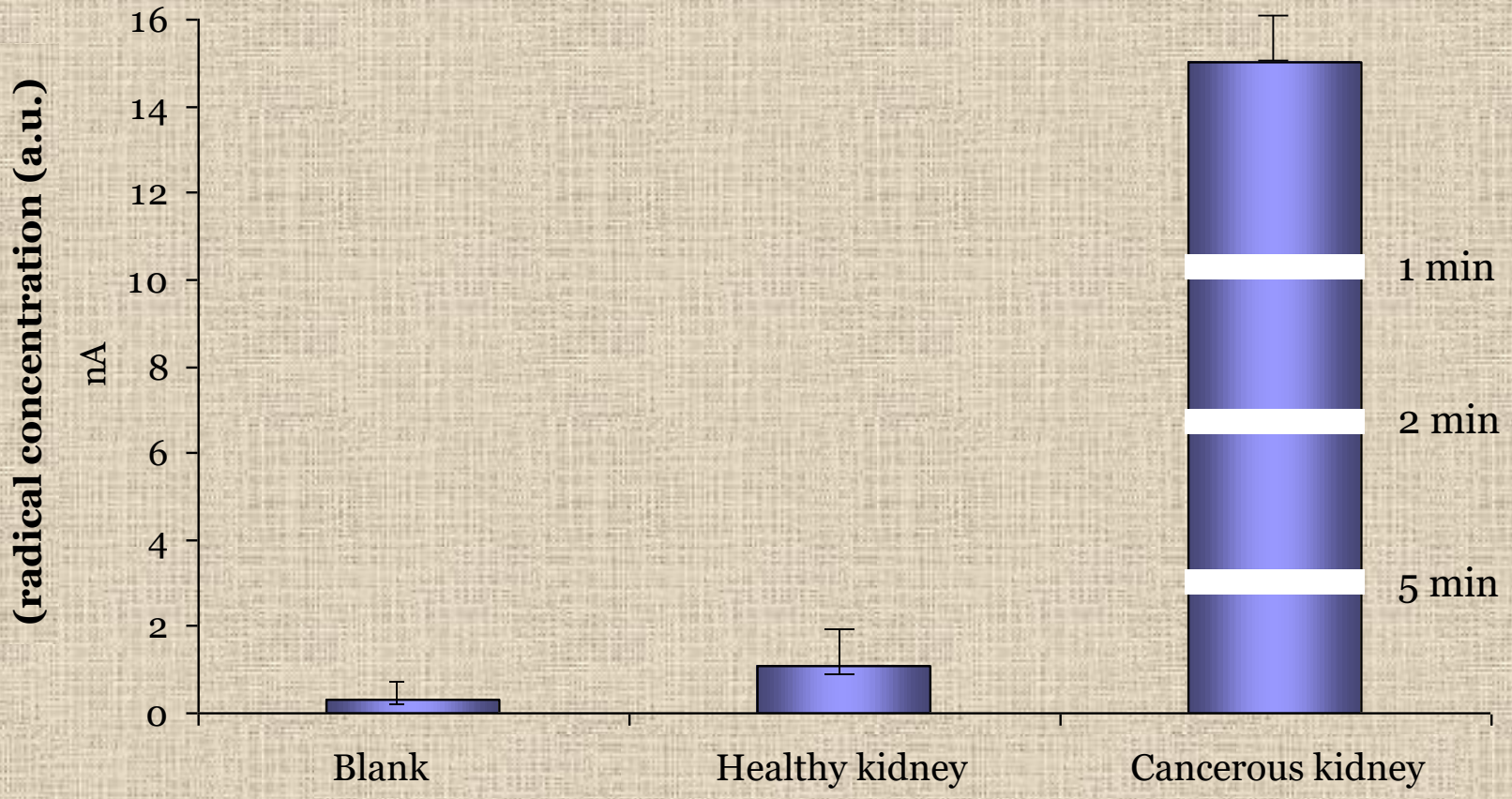
2,40 ± 0,05
(cancerous tissue)

Due to its dioxygenase enzymatic endowment pseudomonas putida is able to biodegrade benzene in the presence of oxygen and the process of oxidative breakdown of the benzene ring occurs as schematized in the following reaction:

$+ 3O_2 + 2NADH + 2H^+$ —(*Pseudomonas Putida*, Fe^{2+})→

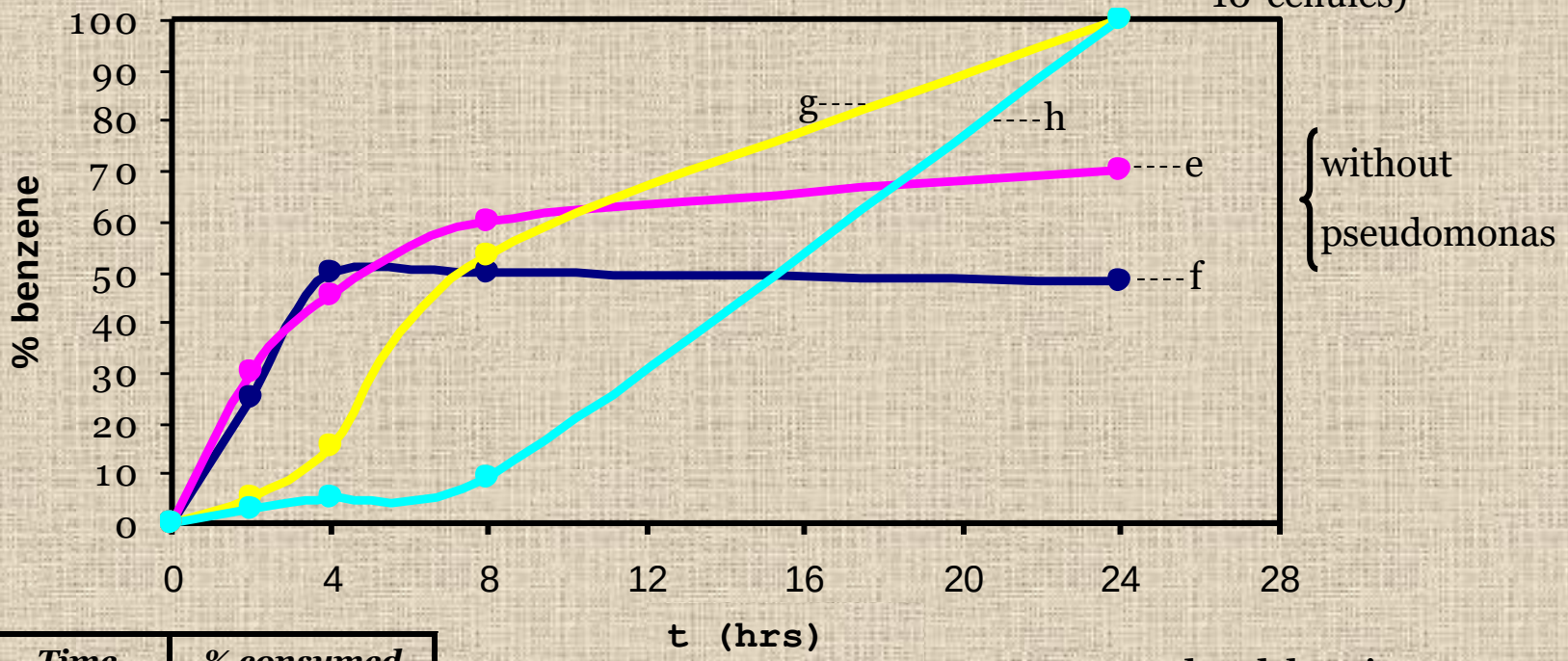
COOH
COOH
$+ 2NAD^+ + 2H_2O$

Effect of antioxidant therapy



T = 37°C

(incubation with pseudom 10^8cellules)



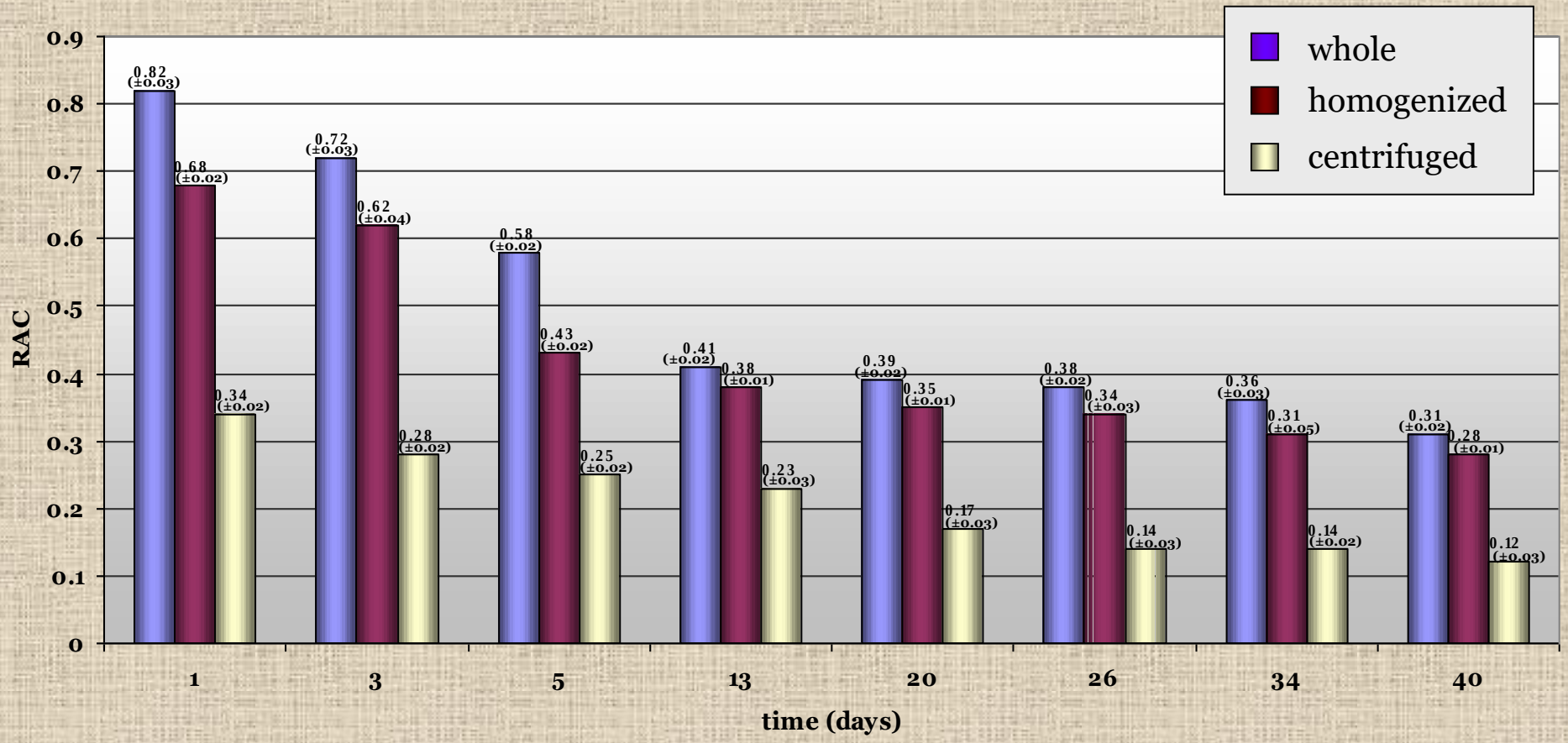
curve	*Time (hrs)*	*% consumed benzene*
g	0	0 ± 2
	4	15 ± 2
	8	54 ± 5
	24	100 ± 2
h	0	0 ± 2
	4	7 ± 2
	8	12 ± 2
	24	100 ± 2

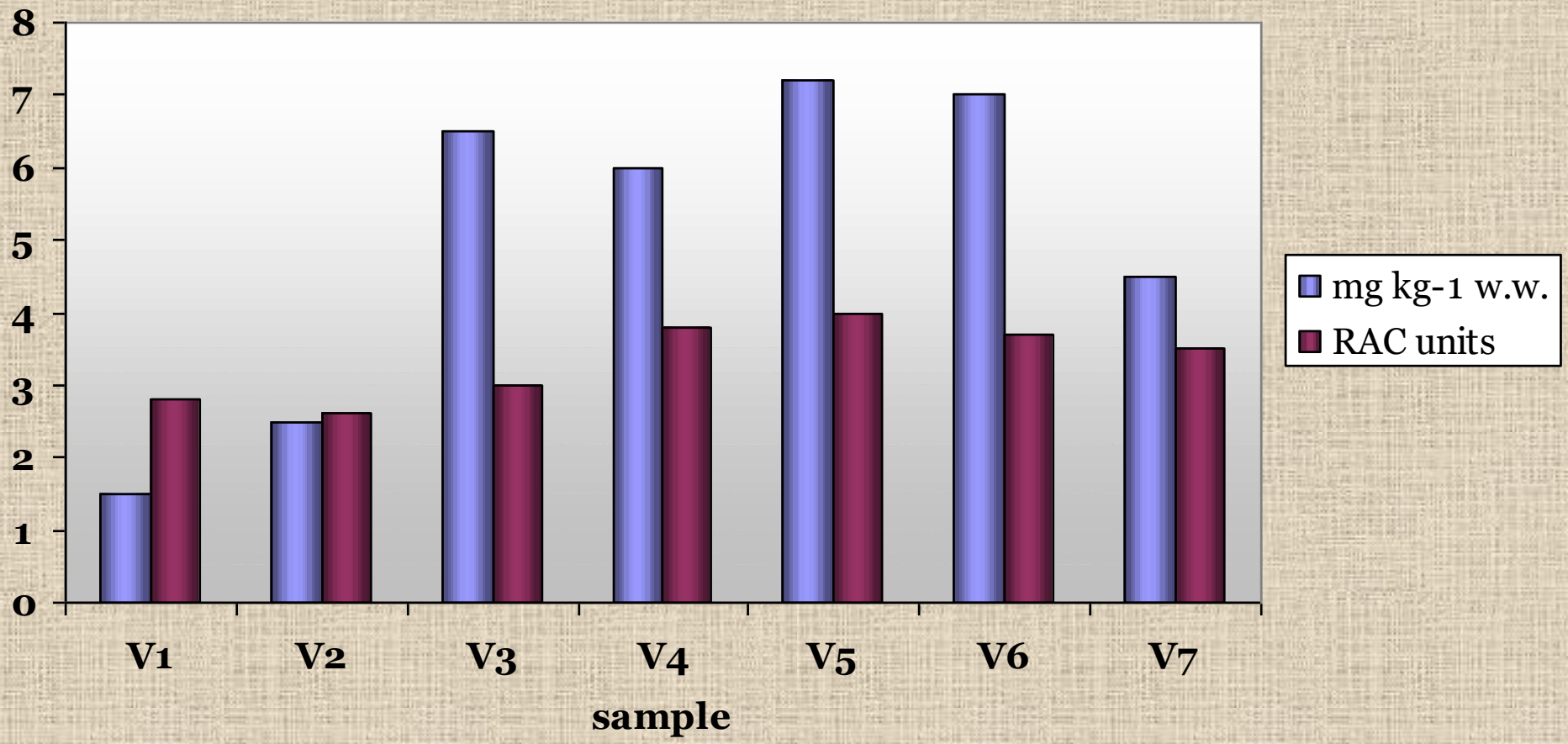
g, e= healthy tissue

h, f= cancerous tissue

Antioxidant capacity of algae

Spirulina subsalsa in colture





Comparison between Mn concentration in molluscs determined by plasma emission spectroscopy and total antioxidant capacity by biosensor.

Role of Fe - SOD
Vs oxidative stress

Incubation with xanthine/xanthine oxidase
(radical generation)

↓

production of SOD

↓

extraction of codifying DNA

↓

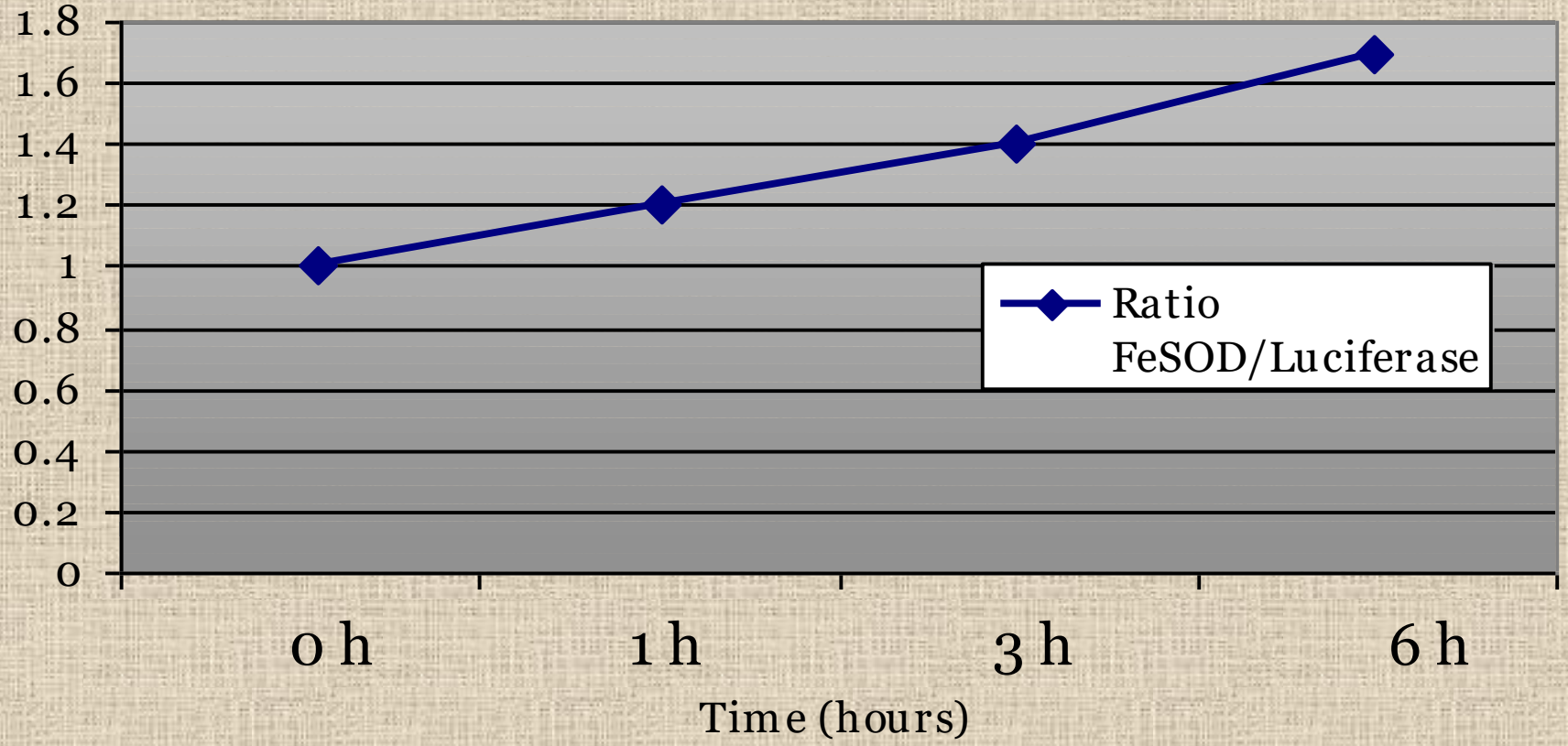
coded DNA

↓

amplification (PCR)

↓

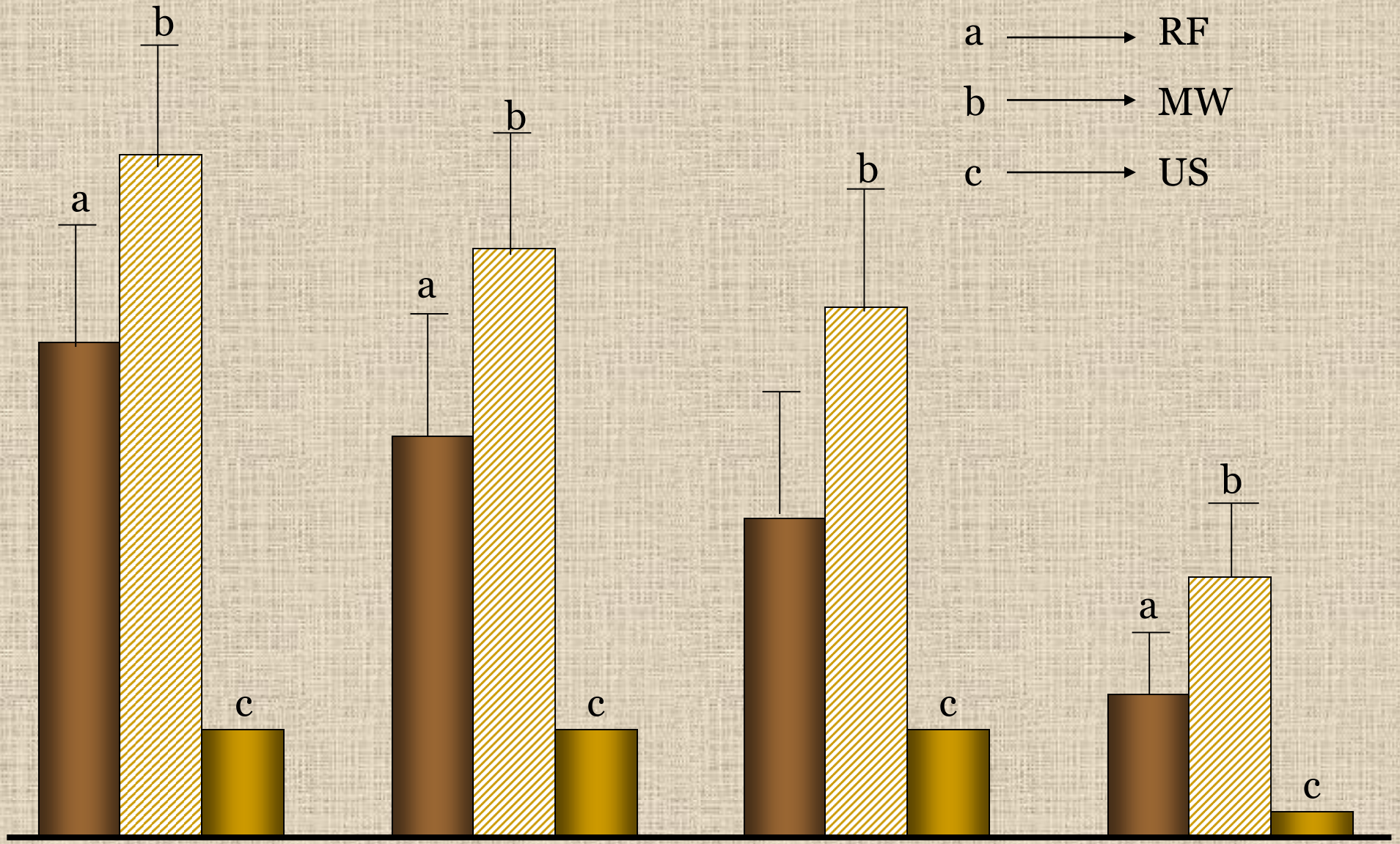
variation in genic expressin of Fe - SOD compared with luciferase used as internal control (its expression is not affected by any in vitro cellular modification).



Graph showing the trend overtime of the ratio of the iron-superoxide dismutase (Fe-SOD) produced by the cultivated alga *Spirulina subsalsa* and luciferase

Production of radicals by MW, RF, US treatment

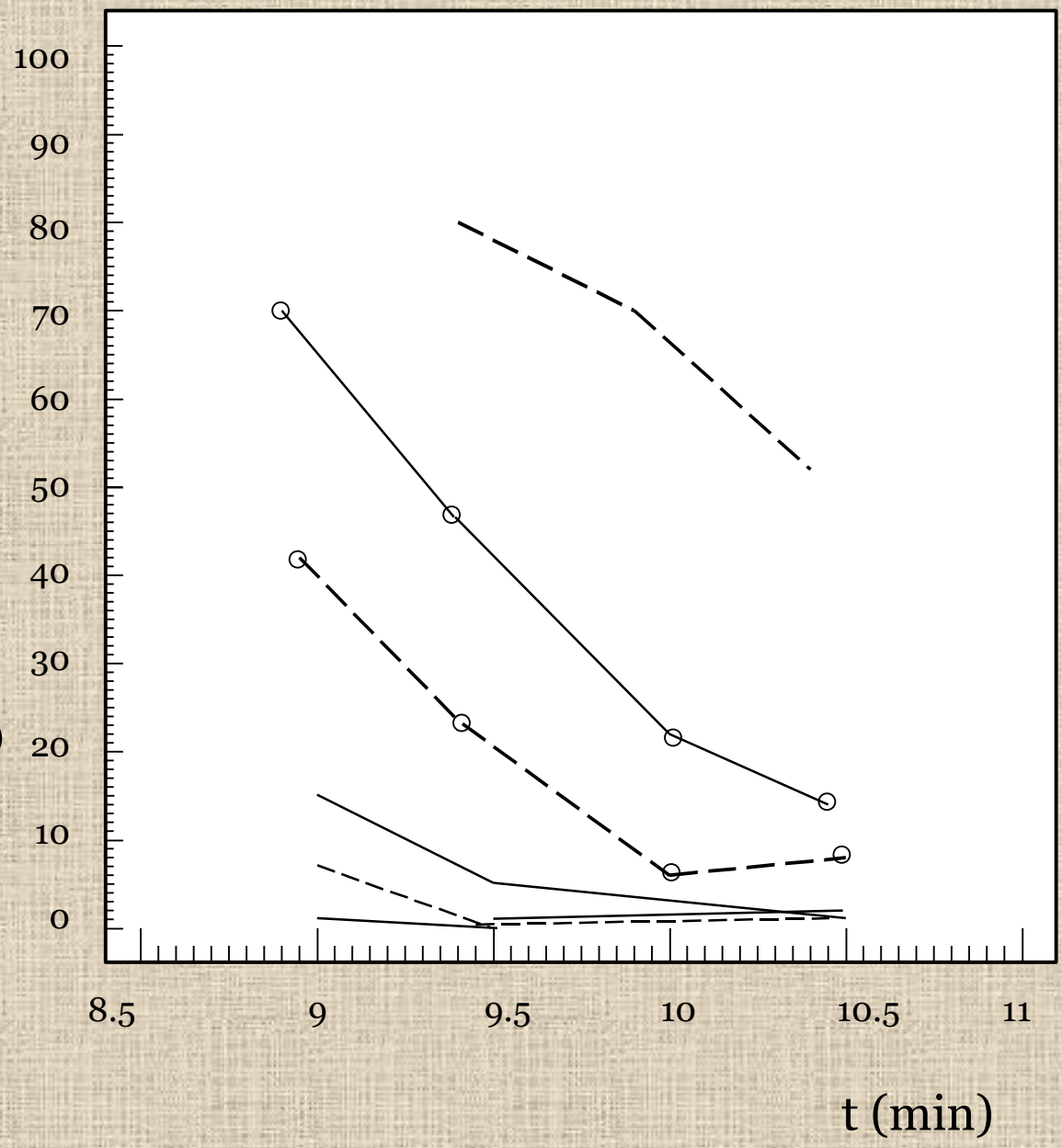
(t = 1 -2 -5 -10 min)



Enzyme inhibition (%) from RF and MW exposure (GOD)

Power	t = 1 m		t = 5 m	
	RF	MW	RF	MW
90W	10	12	28	35
180W	12	15	32	38
360W	20	35	45	86

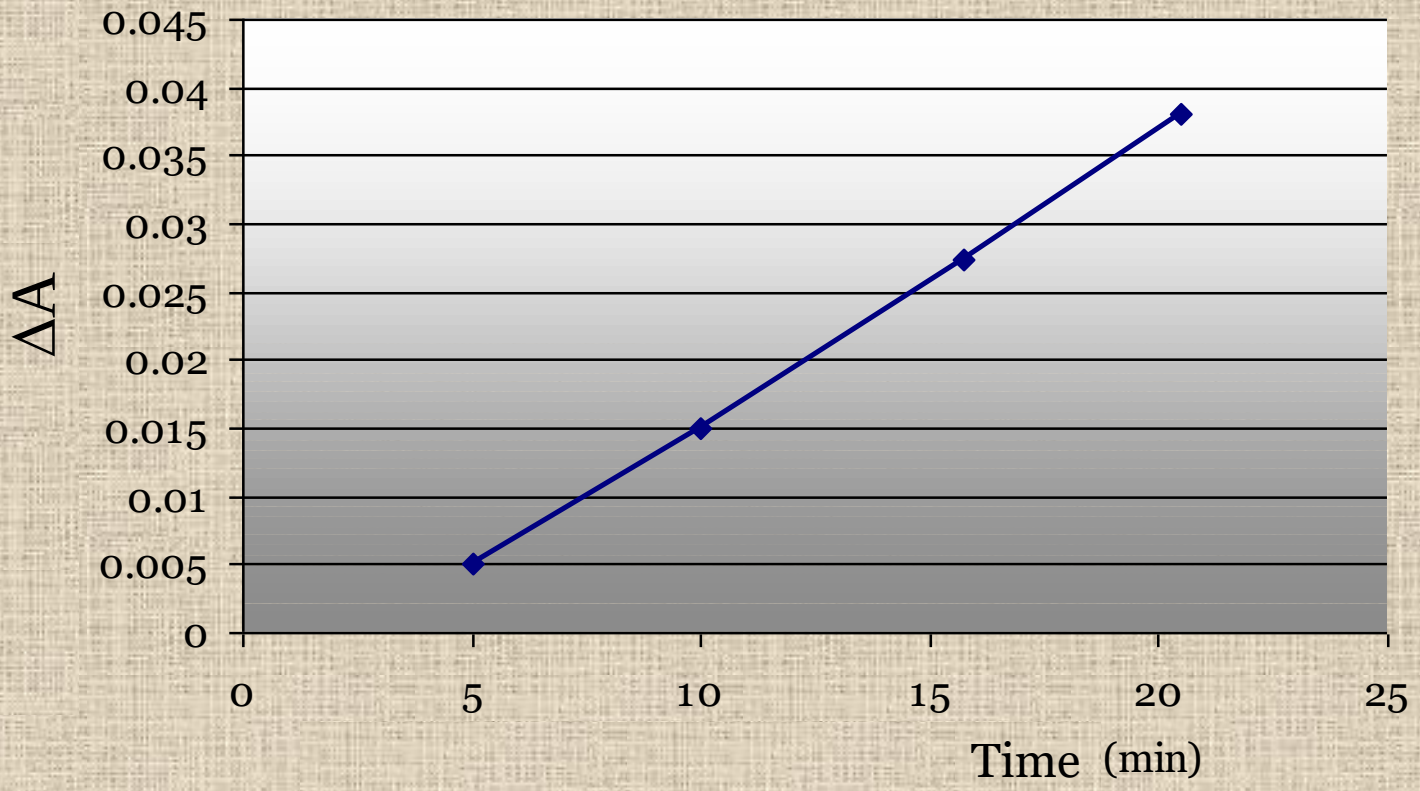
% Degradation of benzene accumulated in human kidney healthy and cancerous tissues (continuous line cancerous, dotted healthy), from up to down: no irradiation, RF (90W), 30 min MW (90W), 30 min



Time	*% inhibition*
0	0
1	0
2	4
2	4
5	8
8	12
10	19
12	20
15	23
18	25
20	28

Inhibition of yeast respiration by exposure to MW for varying times

Radical production on ultrasound treatment (20KHz, 10W/cm²)



$Y = 0{,}0023X - 0{,}0065$

$R^2 = 0{,}97$

Transport of drugs molecules by ultrasounds through synthetic membranes

Dependence on:
nature of membrane
molecular weight of the transported drug
frequency and power of the ultrasounds

If the drug is an antioxidant one (as ascorbic acid, cysteine, vitamin E) the transported amount results to be less than expected probably due to a consumption on reaction with the radicals produced by the ultrasounds.

Antioxidant properties and compound determination methods

Folin Ciocolteau

Aldheyde/Acid inhibition

Malonaldheyde

Rancimat test

Deoxyribose

Superoxide dismutase enzyme

TEAC (troloc equivalent antioxidant capacity)

ORAC (oxygen radical absorbance capacity)

FRAP (iron ions)

Galvinoxil

Synthetic antioxidants

Problems of safety

Pharmaindustry and Food industry

Natural origin antioxidants contained in many matrices such as tea (catechins), coffee, (polyfunctional acids), carrots (antocyans), oil, fruits, vegetables

Extraction methods

looking for

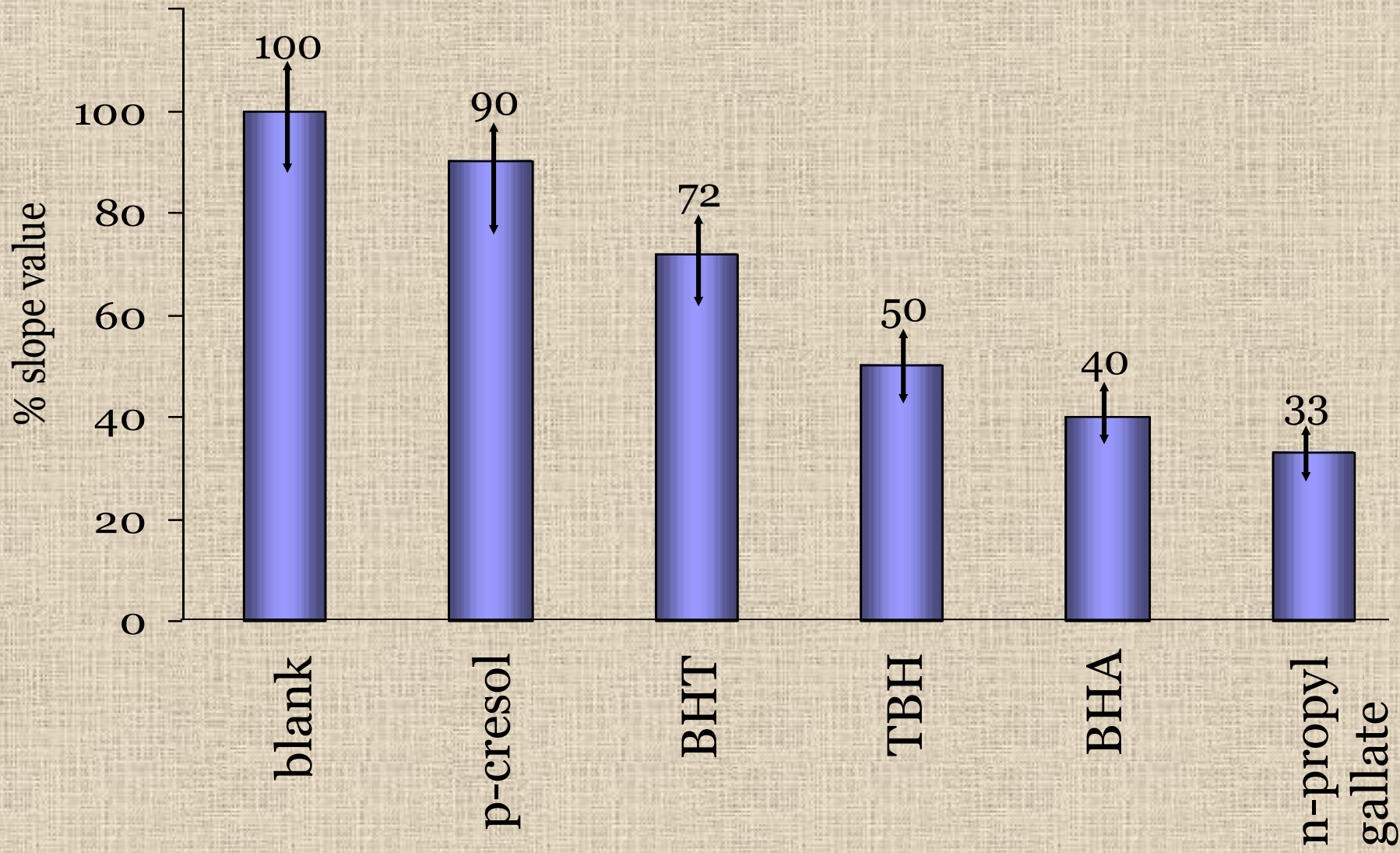
No solvent residues

high purity

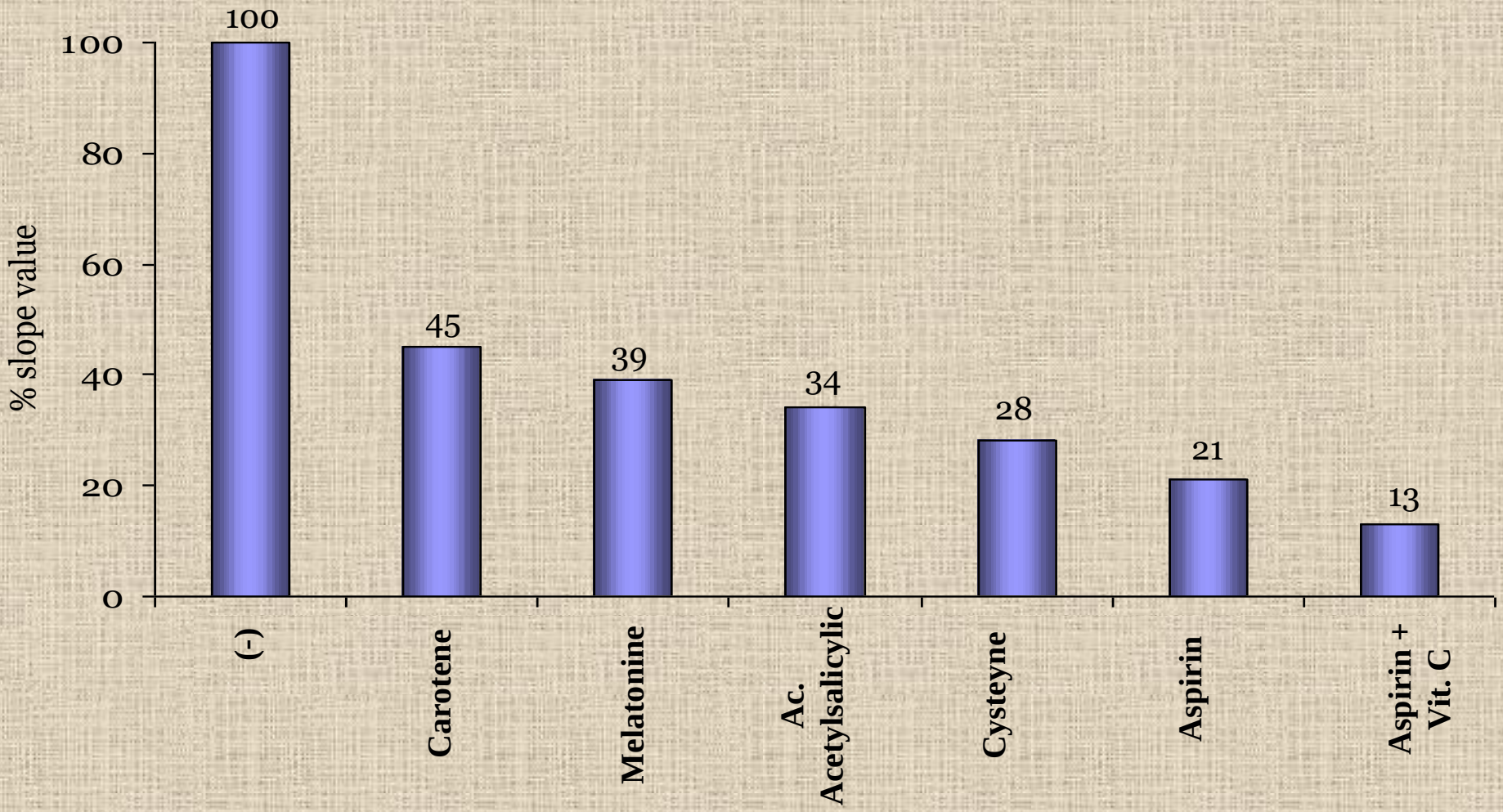
+

high recovery

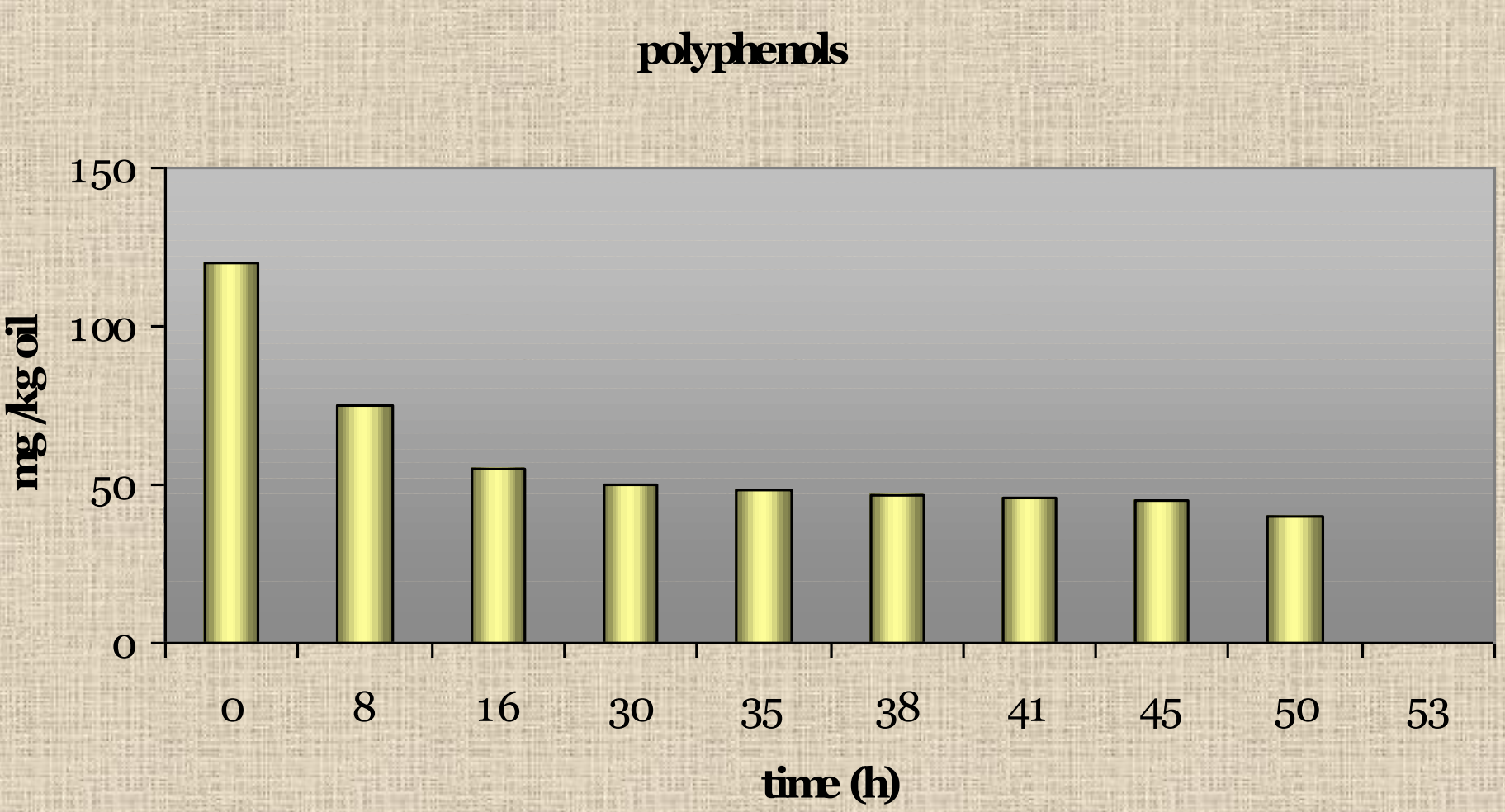
Supercritical fluid (CO_2)



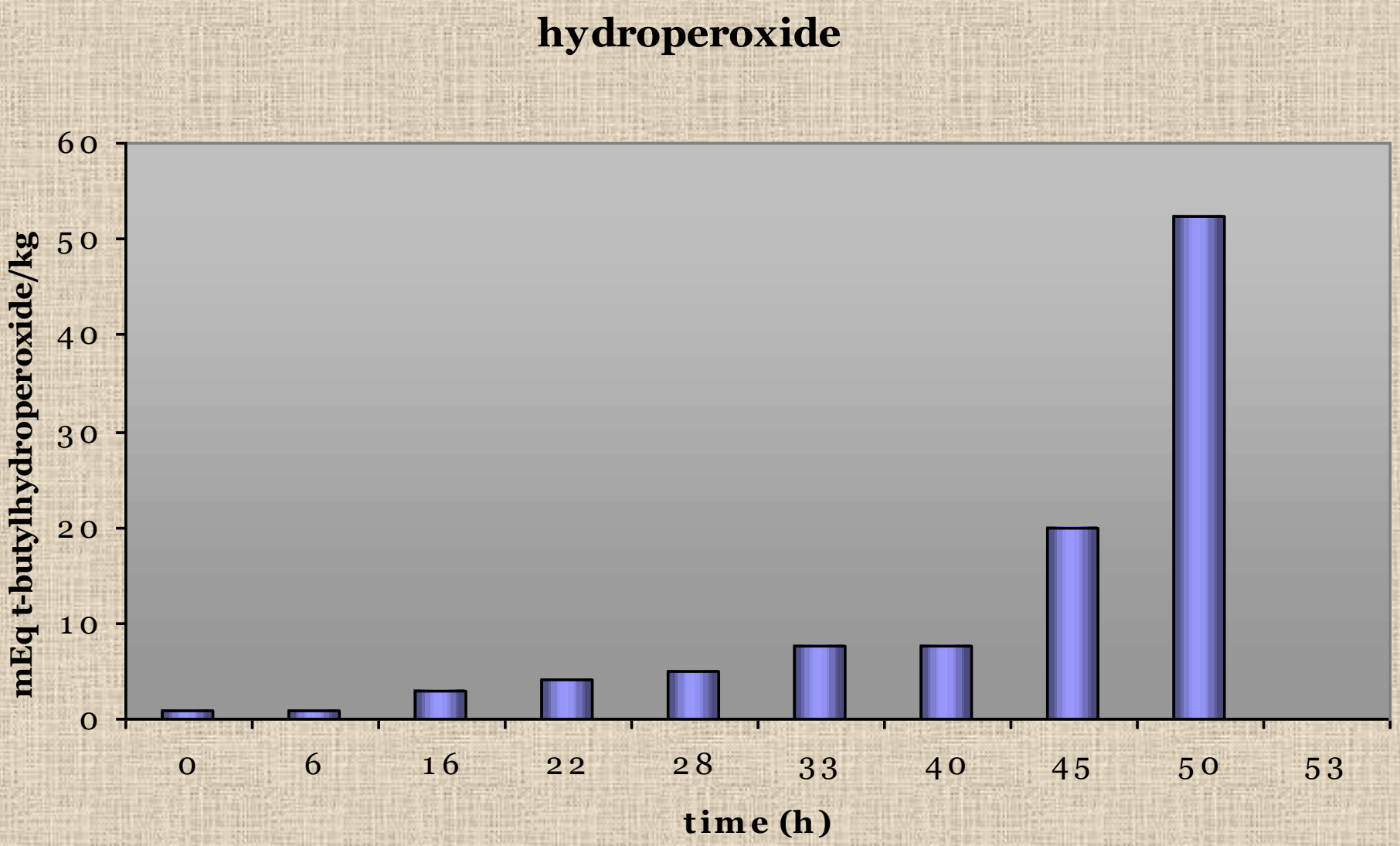
BHT: butyl hydroxytoluene; TBH: tert-butyl hydroquinone; BHA: butyl hydroxyanisole.



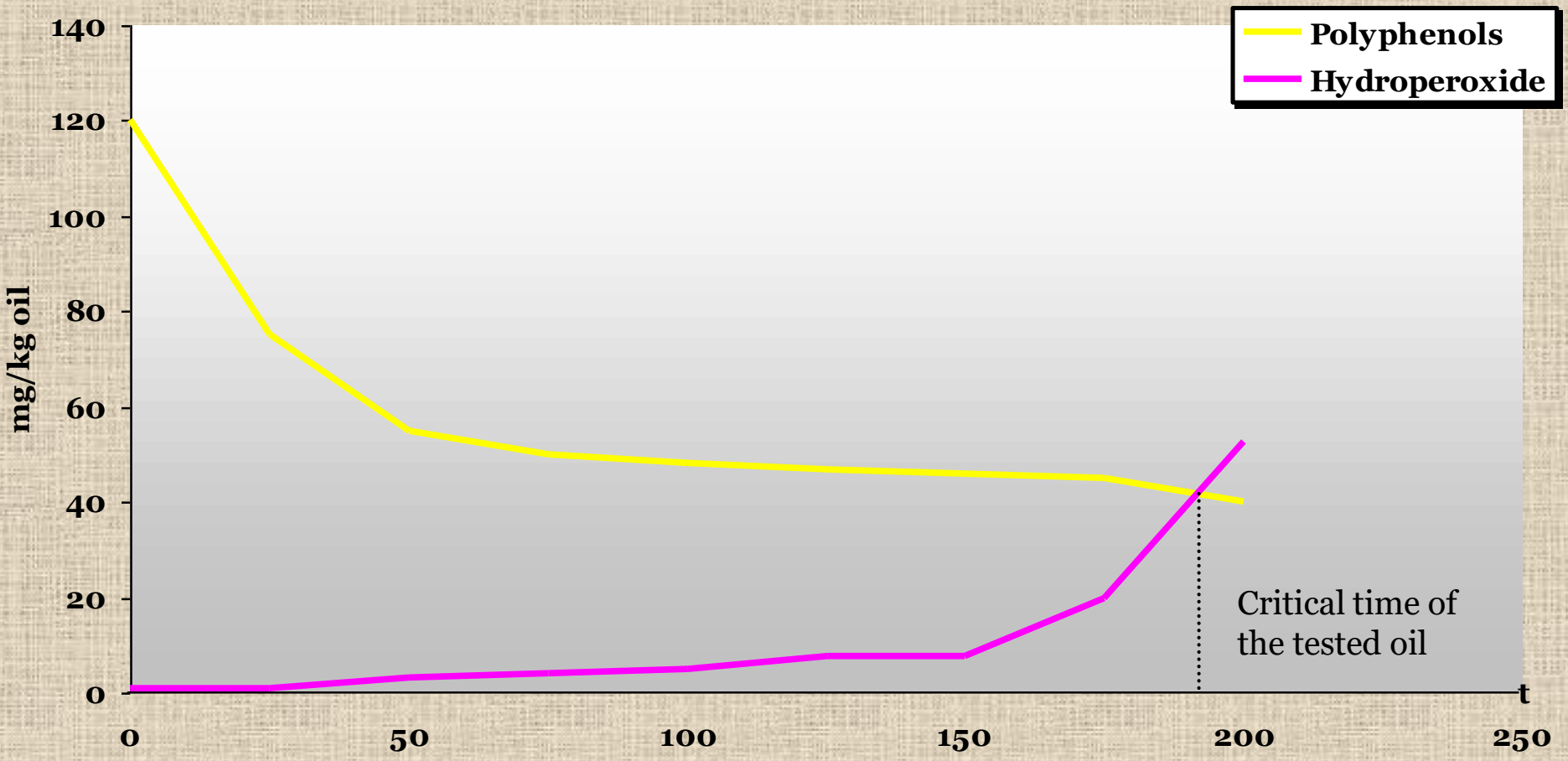
Evaluation of scavenger properties of several compounds and drugs using the SOD/H_2O_2 biosensor working in aqueous phosphate buffer solution 0.05 M at pH=7.5. Behaviour of the % sensitivity if the biosensor in absence (-) and in presence of different antioxidant compounds; β-carotene and melatonine at saturated solutions; all the other ones at 0.05 M concentration.

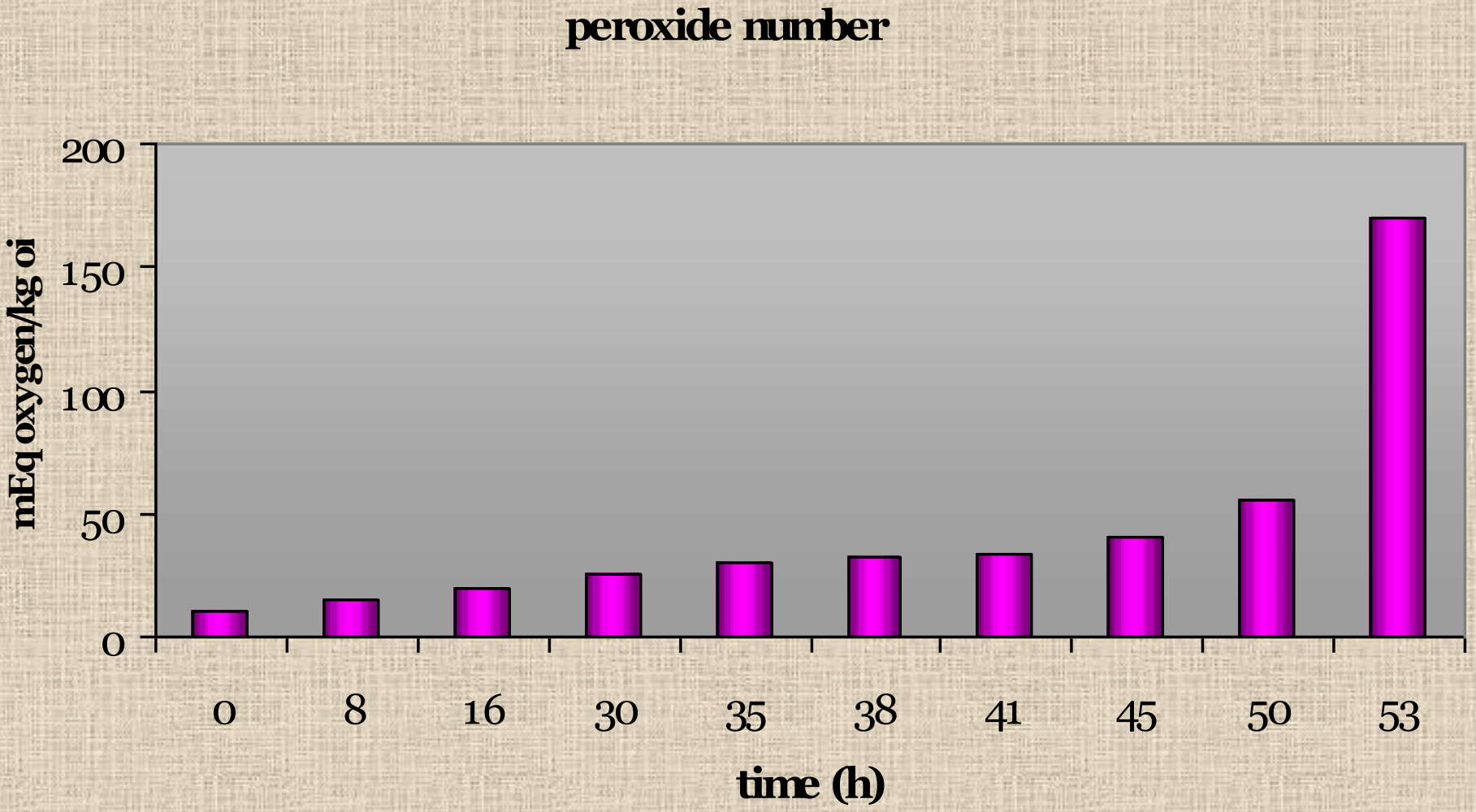


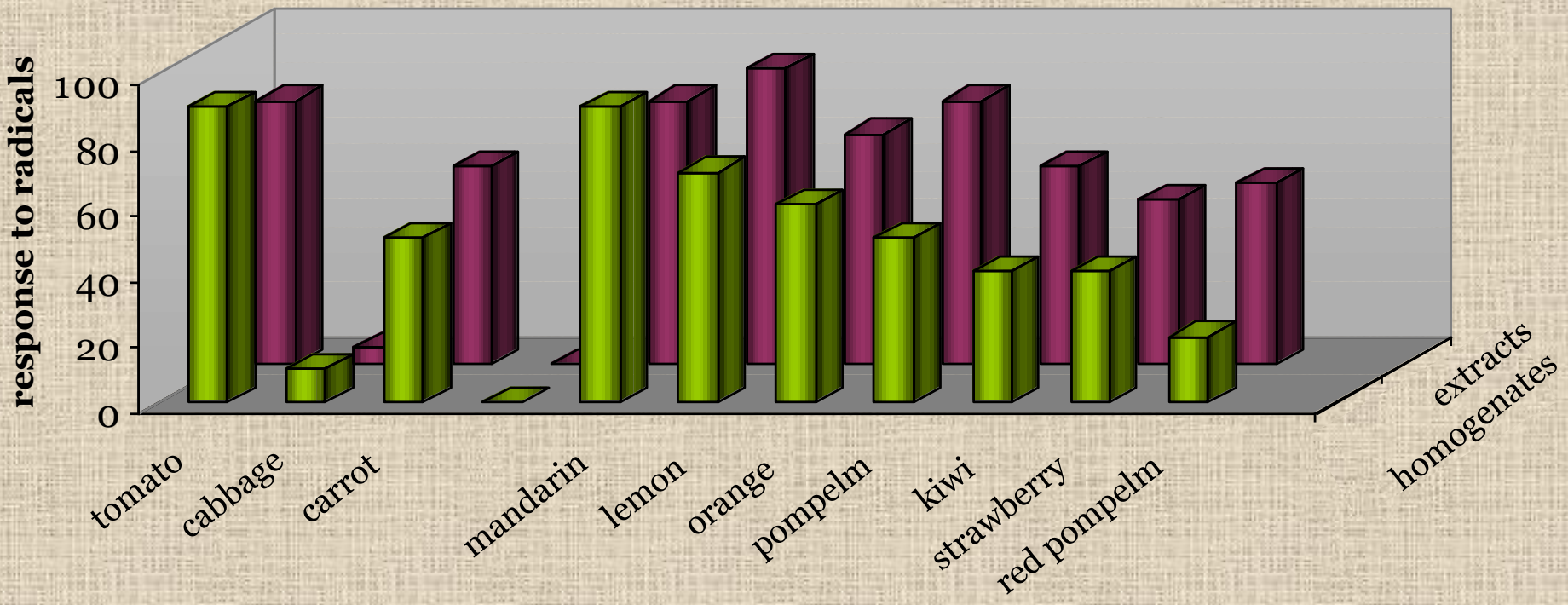
Trends of considered quality parameters during the process of artificial rancidification of extra virgin olive oil.

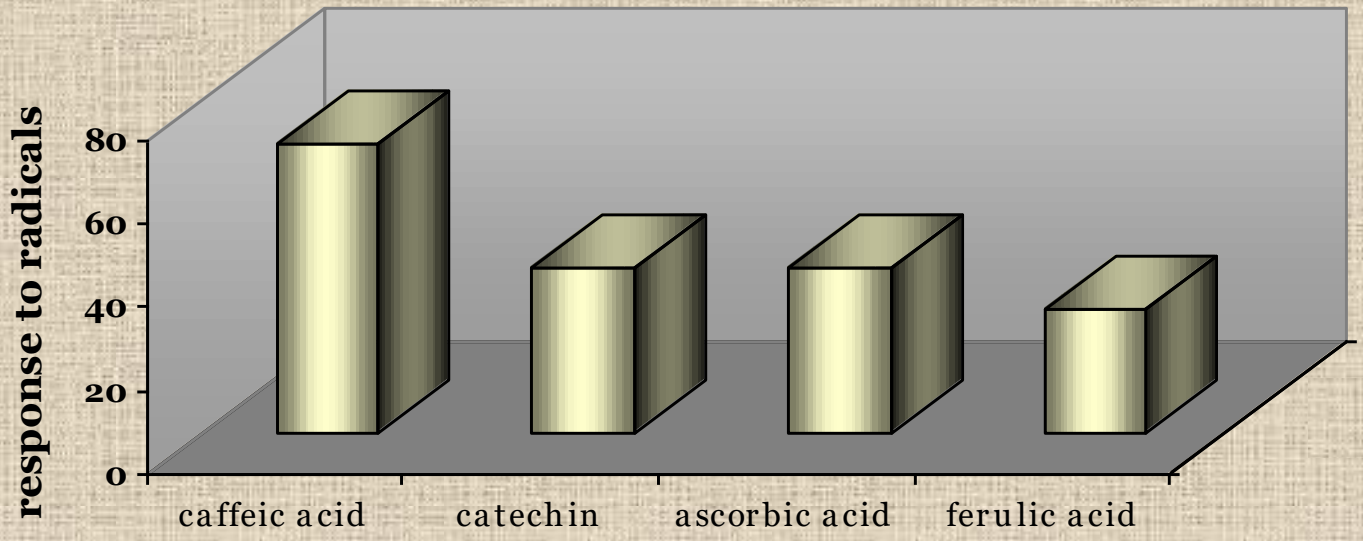


(artificial rancidification)

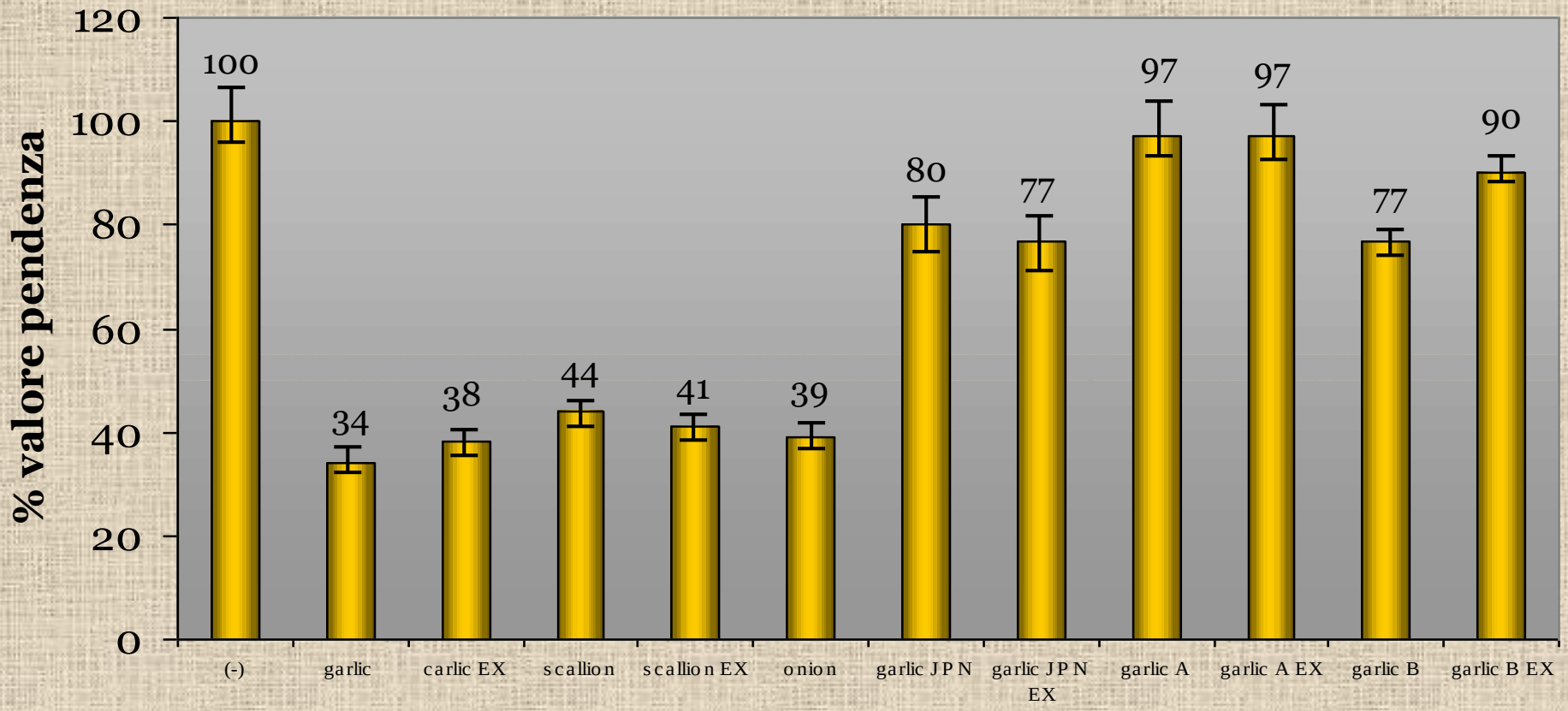

Polyphenols
Hydroperoxide
mg/kg oil
140
120
100
80
60
40
20
0
0
50
100
150
200
250
t
Critical time of
the tested oil


peroxide number
mEq oxygen/kg oi
200
150
100
50
0
0
8
16
30
35
38
41
45
50
53
time (h)


response to radicals
100
80
60
40
20
0
tomato
cabbage
carrot
mandarin
lemon
orange
pompelm
kiwi
strawberry
red pompelm
extracts
homogenates


response to radicals
80
60
40
20
0
caffeic acid
catechin
ascorbic acid
ferulic acid

Antioxidant properties of different commercial products based on garlic



Ex = aqueous extracts

Antioxidant	Antioxidant capacity (anodic area)				r_{max}
	[Copper salt] = 0	$[Cu(NO_3)_2 \bullet 3H_2O]$ = 10^{-2}mol/L	$[CuSO_4] = 10^{-2}$ mol/L	$[CuCL_2 \bullet 2H_2O]$ = 10^{-2}mol/L	
Ascorbic acid	1.00	6.343	4.693	3.959	6.343
Glutathione	0.298	2.210	1.127	5.652	18.966
n -propilgallate	1.822	7.921	7.045	11.063	6.071
trolox	1.020	2.948	5.793	1.694	5.679

antioxidant	**Logβ**	**prevailing equilibria**
Ascorbic acid H_2L	14,58 ± 0,40	$Cu^{2+} + 3\ HL^- \rightleftharpoons Cu(HL)_3^-$
Glutathione H_3L	11,64 ± 0,28	$Cu^{2+} + 2\ H_2L^- \rightleftharpoons Cu(H_2L)_2^-$
n-propilgallate H_3L	18,0 ± 2,0	$Cu^{2+} + 4\ H_3L^- \rightleftharpoons Cu(H_3L)_4^-$
Trolox H_2L	10,64 ± 0,20	$Cu^{2+} + 3\ HL^- \rightleftharpoons Cu(HL)_3^-$