Radical scavenging capacity of melanoidins isolated from Maillard reaction mixtures

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AIM

Study of the kinetic behaviour of the MR mixtures and melanoidins as free radical scavengers in a hydrophilic media.

Maillard Reaction Mixtures

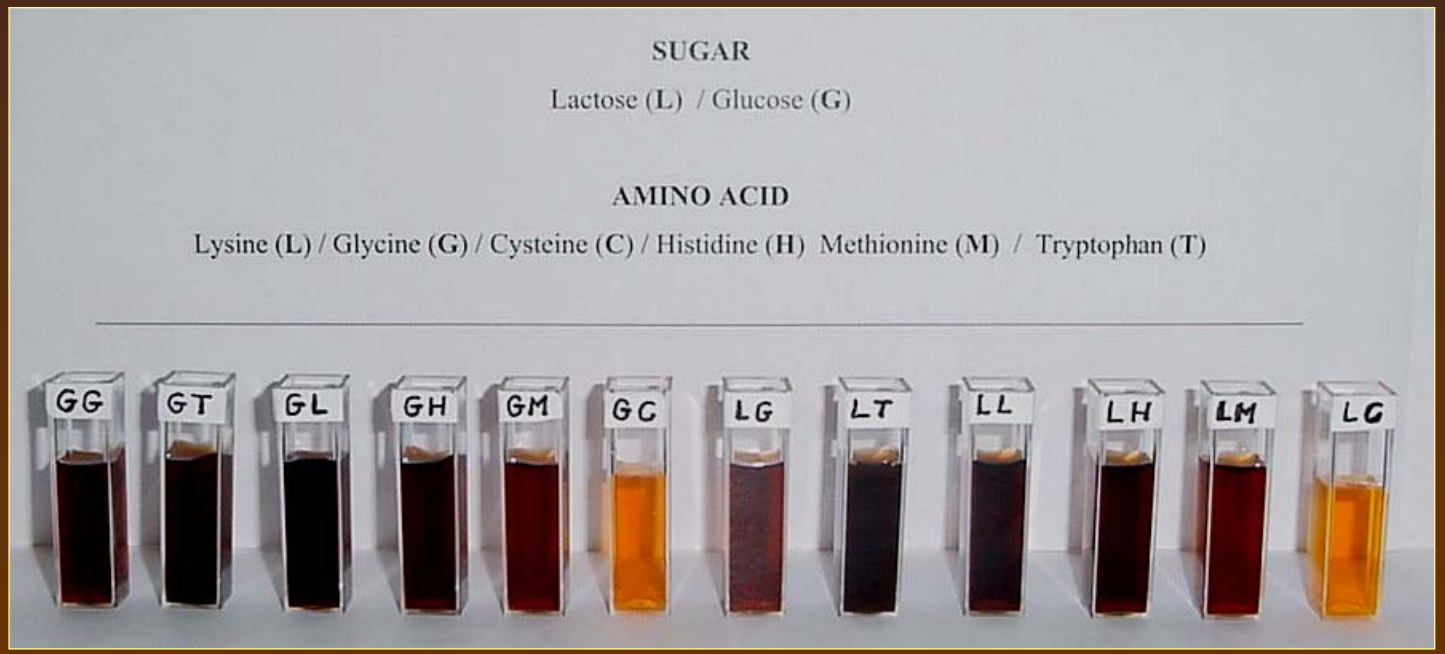
Sugars	Amino acids
Glucose	Glycine
Lactose	Histidine
	Lysine
	Tryptophan
	Cysteine
	Methionine

0.1 M Na-PO_4 Buffer (pH 7.0)

100ºC/24 h (250 mL flasks)

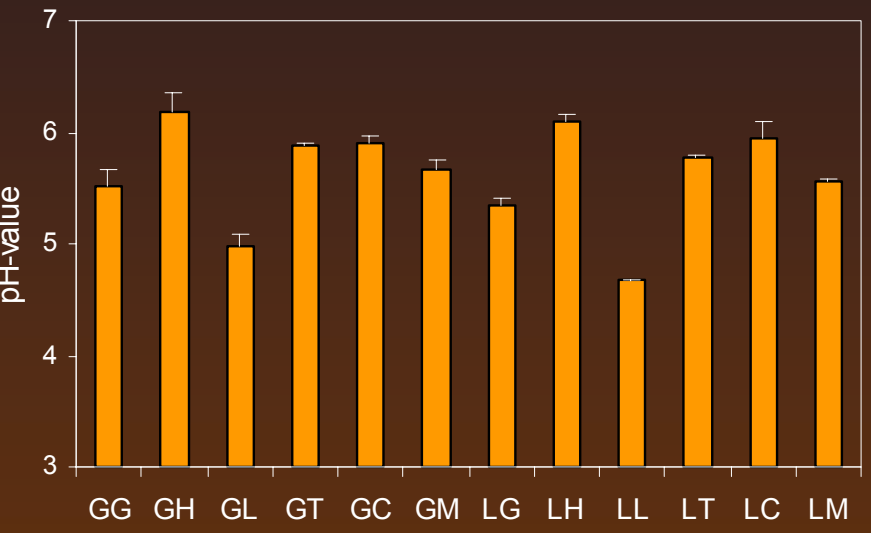
without pH control

Rate 1:1 / 0.1M

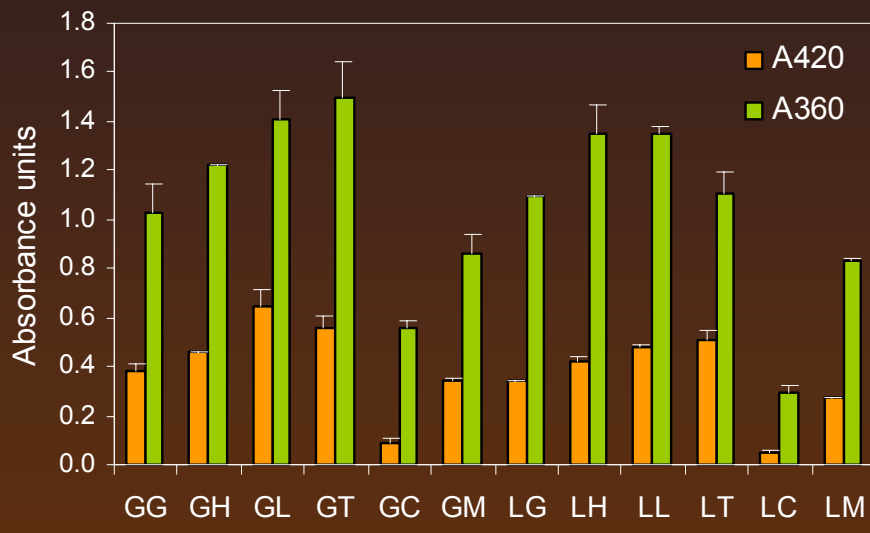


Maillard Reaction Mixtures

pH-value



Browning



Coffee Brew

COST STANDARD PROCEDURE

100 g coffee powder + 300 mL hot water (75ºC)

↓

Stirring
(5 min)

↓

Filtration
(Whatman n.4)

↓

Defatted with dichloromethane
(2x200 mL)

↓

Coffee sample

pH = 4.78

A_{420} (1/100) = 1.328

A_{360} (1/100) = 3.436

Melanoidins

MR Mixtures / Coffee

↓

Ultrafiltration

Amicon Unit, Millipore
10kDa nomimal molecular mass cut-off
membrane of regenerated cellulose
at least three cycles

↓

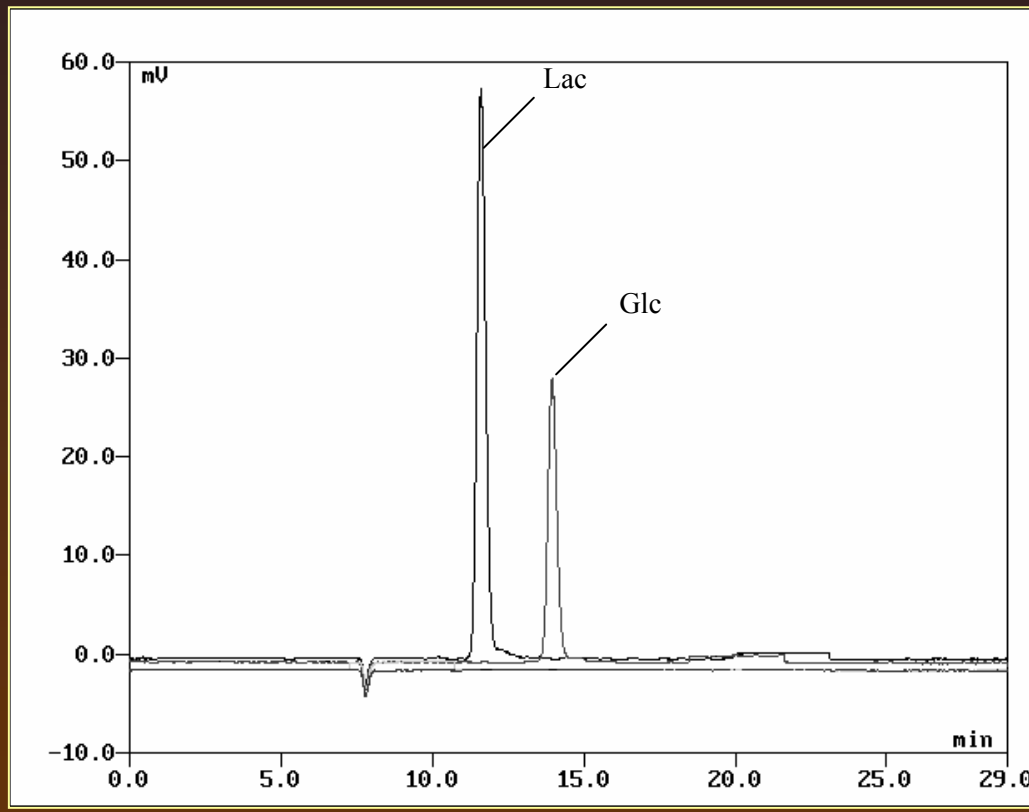
Dry-vacuum

↓

Melanoidin

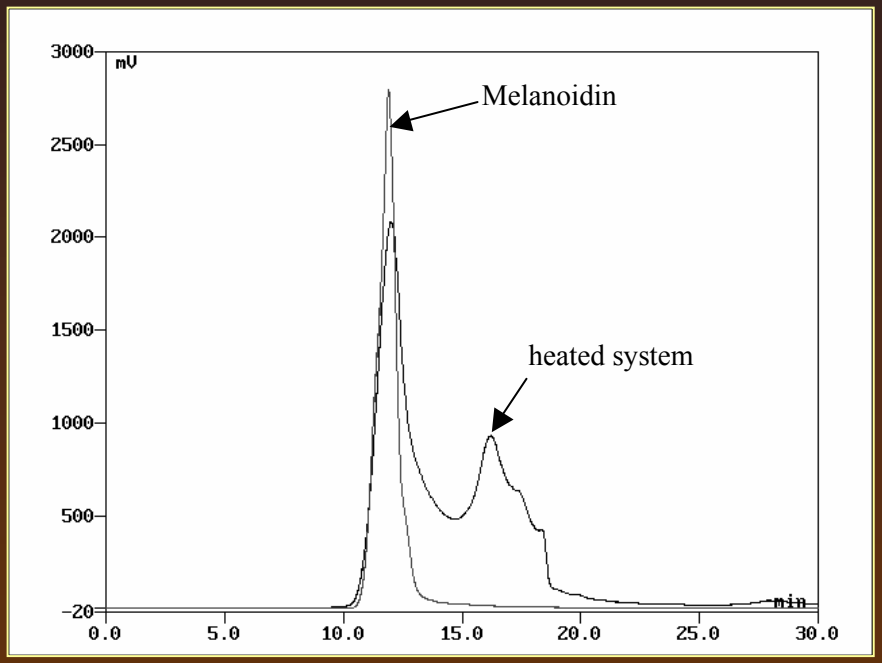
High molecular mass material

IE-HPLC (LG, GG systems)

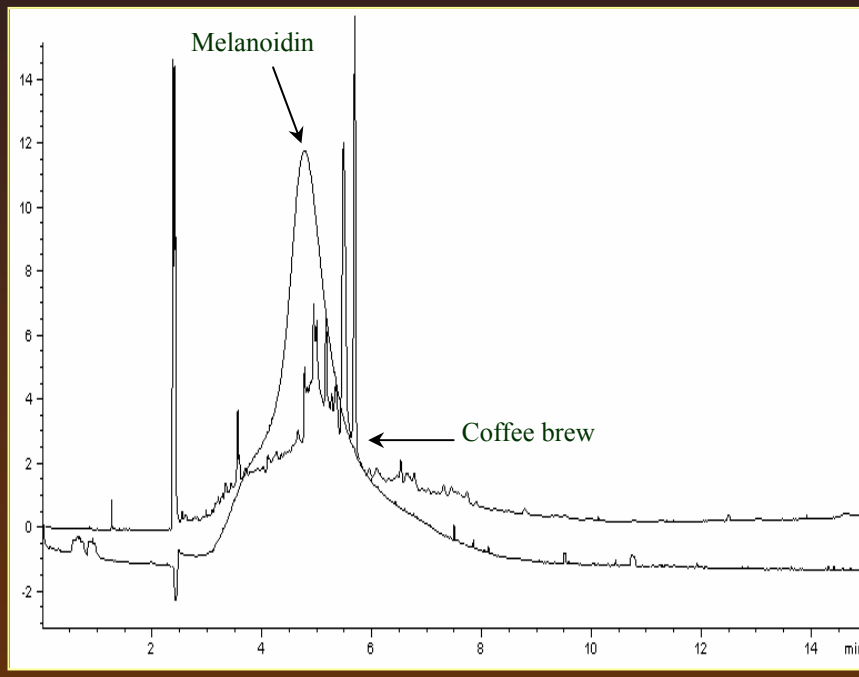


Melanoidins

HPGPC (GG system)



CZE (Coffee)



DMPD method

N,*N*-dimethyl-*p*-phenylenediamine
(DMPD)

H^+ + Oxidant

- Hydrogen peroxide
- Copper chloride
- ABAP
- ferric chloride
- sodium hipochloride

$DMPD^{\cdot+}$
(purple)

AOH

AO

$DMPD^+$
(uncolourless)

Fogliano et *al.*(1999)

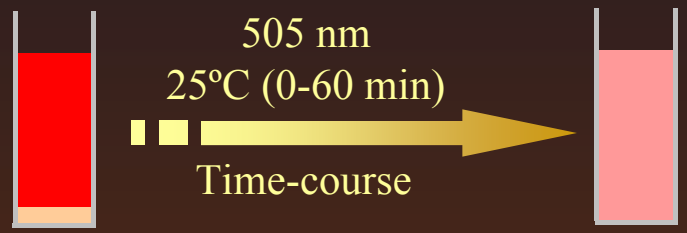
$FeCl_3$

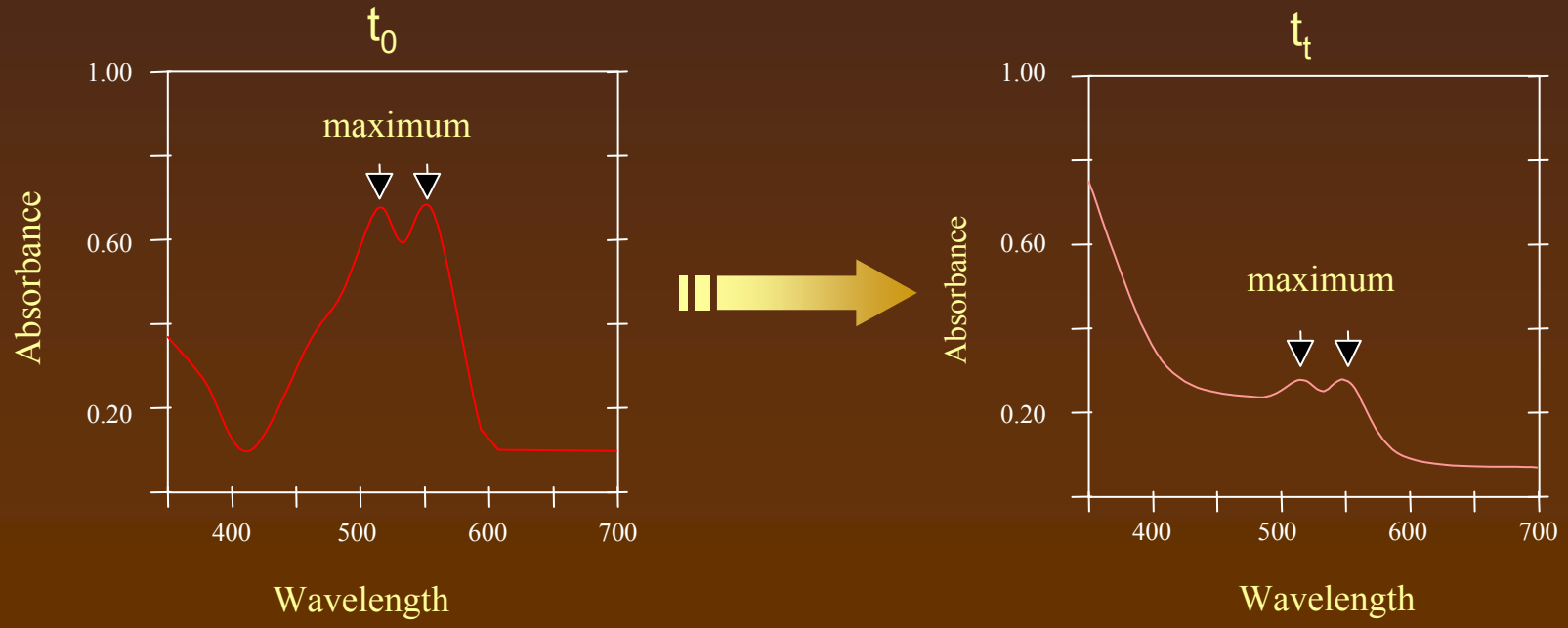
10:1 / DMPD:Fe^{3+}

DMPD method for MR mixtures and melanoidins

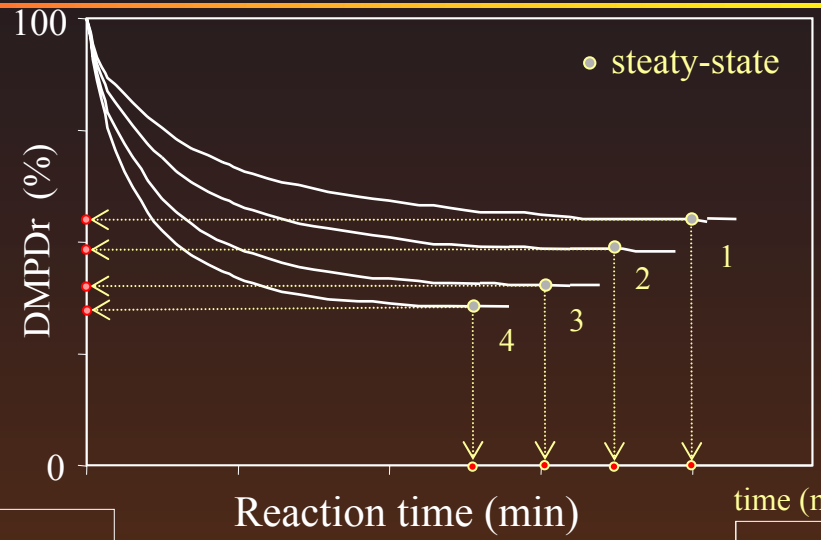
0.75 mM DMPD·$^{+}$ solution (1 mL)
Na-Ac 0.1M, pH 5.25, $FeCl_3$ 0.1 mM

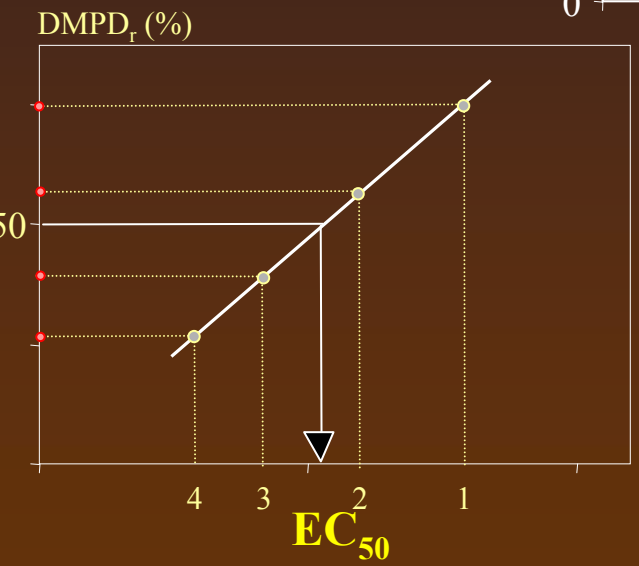
Sample (100 µL)

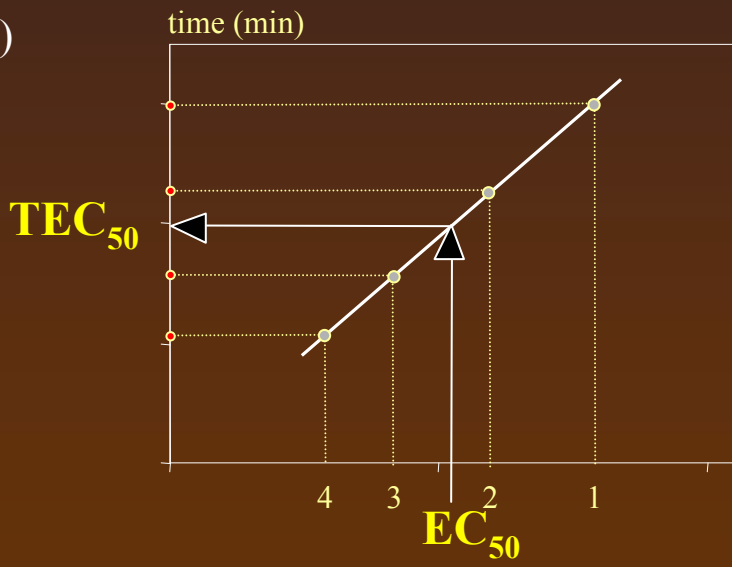




Kinetic approach







EC_{50}: dilution factor of a MR mixture necessary to decrease the initial A-505 of $DMPD^{\cdot+}$ by 50% at the steady-state

TEC_{50}: time (min) necessary to reach the steady-state at the EC_{50} concentration

Kinetic approach

Antiradical Efficiency Concept

MR Mixtures

$$AE = \frac{EC_{50}\ \text{(dilution factor)}}{TEC_{50}\ \text{(min)}}$$

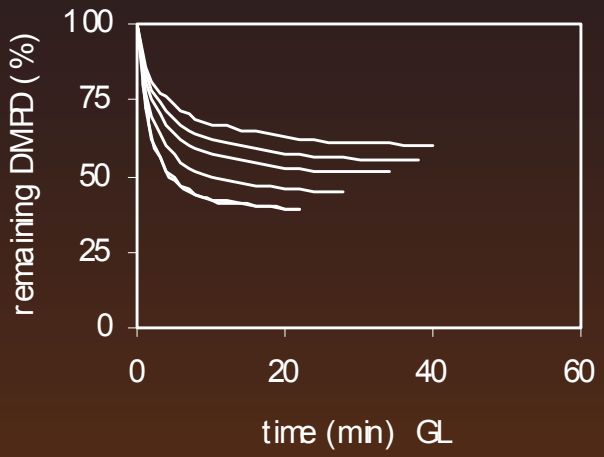
Melanoidins

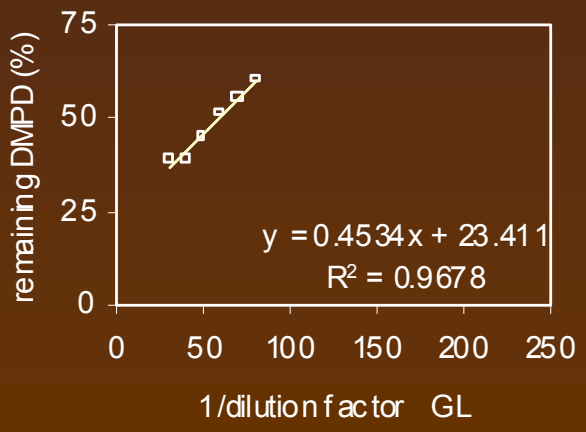
$$AE = \frac{1}{EC_{50}\ \text{(mg/mL)} \cdot TEC_{50}\ \text{(min)}}$$

Sanchez-Moreno et *al.*(1998)

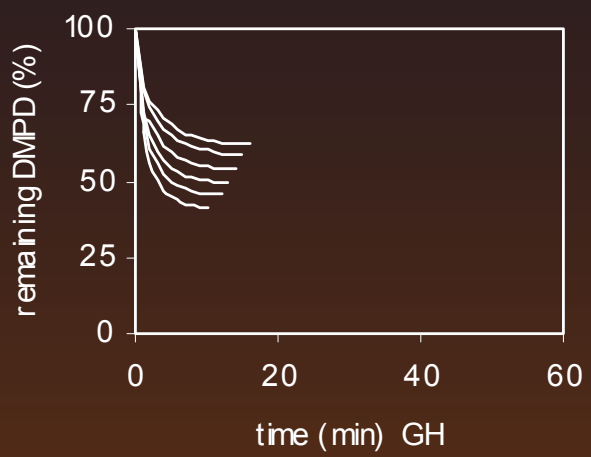
Kinetic approach

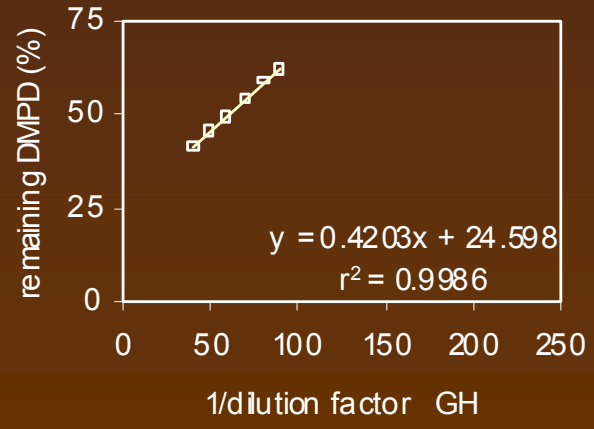
2-GL-I



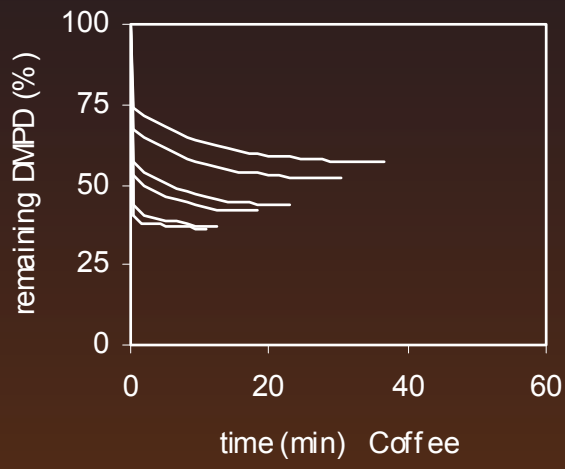


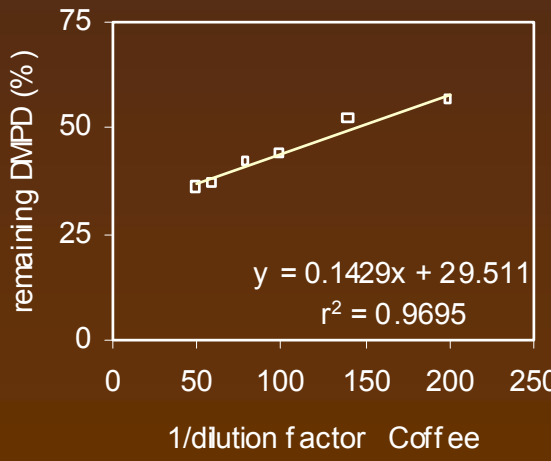
1-GM-II





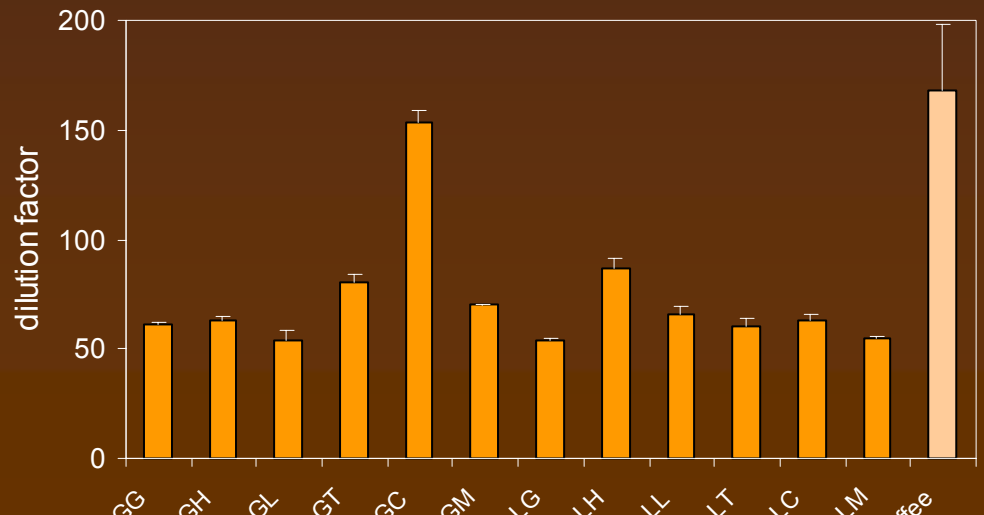
1-Coffee-III



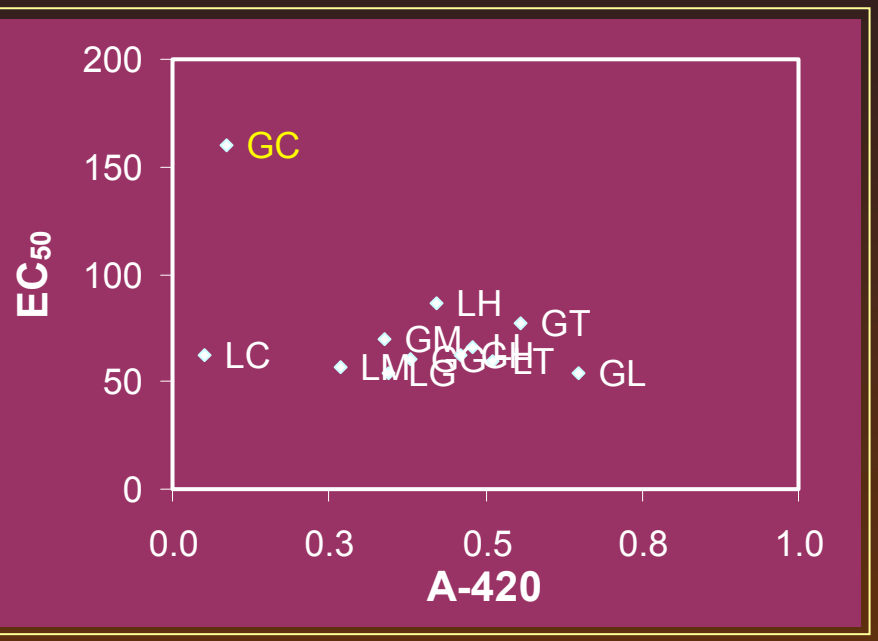


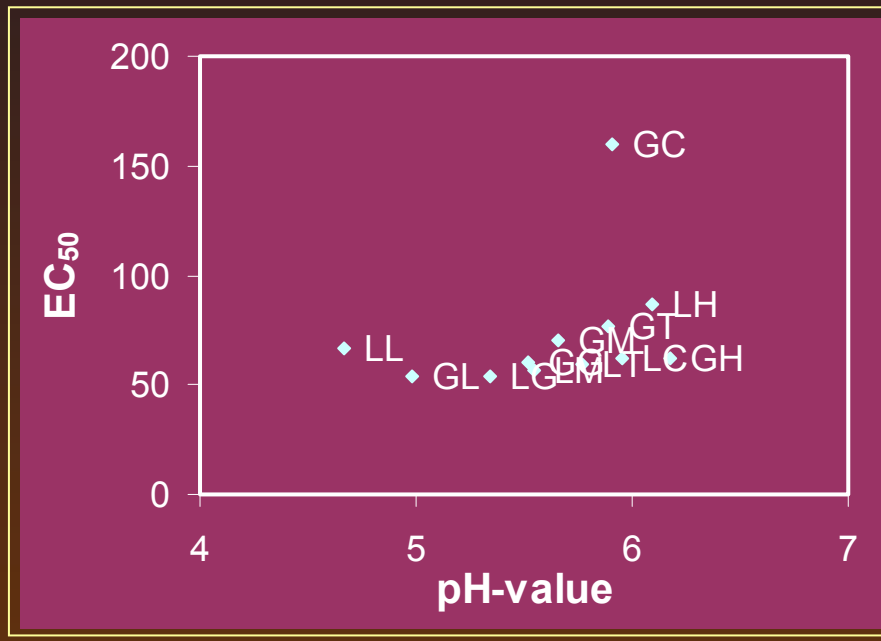
EC_{50}

System	a	b	r2	EC 50	dilution range	n
GG	2.371	-58.150	0.985	60.4	(20-80)	24
GH	2.618	-68.574	0.989	62.3	(40-100)	12
GL	2.386	-65.370	0.994	53.9	(30-80)	24
GT	2.627	-54.482	0.998	76.9	(30-100)	23
GC	4.549	-67.849	0.904	159.6	(20-220)	24
GM	2.345	-47.244	0.988	70.0	(20-100)	24
LG	1.876	-39.669	0.984	54.1	(20-70)	24
LH	3.354	-81.000	0.984	86.7	(20-110)	23
LL	2.904	-78.753	0.985	66.5	(30-100)	24
LT	2.710	-75.772	0.996	59.7	(40-100)	24
LC	2.467	-61.245	0.986	62.1	(40-100)	24
LM	2.825	-84.713	0.964	56.5	(20-100)	24
Coffee	7.472	-208.026	0.958	165.6	(50-200)	18



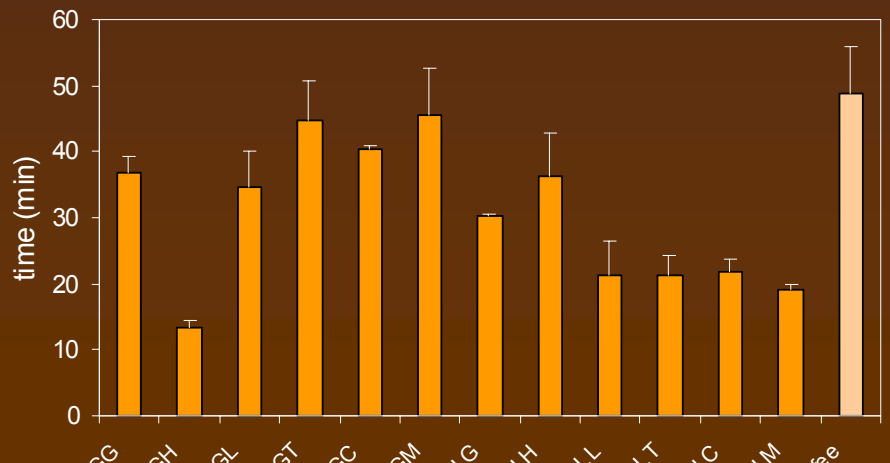
EC_{50}


200
150
100
50
0
EC50
GC
LH
GT
GM
LC
LM
LG
GL
0.0
0.3
0.5
0.8
1.0
A-420

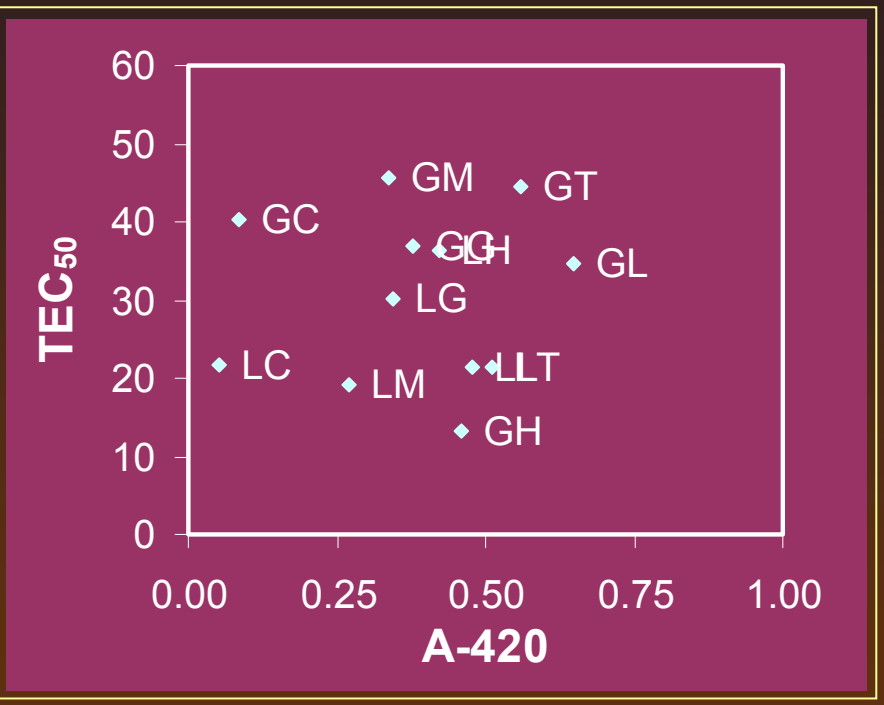

200
150
100
50
0
EC50
GC
LH
GT
LL
GL
LG
GH
4
5
6
7
pH-value

TEC_{50}

System	a	b	r2	EC50 dil.fac	TEC50 min	dilution range	n
GG	0.338	15.057	0.963	60.4	**35.5**	(20-80)	24
GH	0.121	5.575	0.981	62.3	**13.1**	(40-100)	12
GL	0.381	13.311	0.966	53.9	**33.8**	(30-80)	24
GT	0.124	30.918	0.758	76.9	**40.5**	(30-100)	23
GC	0.056	26.690	0.317	159.6	**35.7**	(20-220)	24
GM	0.150	27.413	0.579	70.0	**37.9**	(20-100)	24
LG	0.394	8.873	0.993	54.1	**30.2**	(20-70)	24
LH	0.100	23.074	0.479	86.7	**31.7**	(20-110)	23
LL	0.183	23.998	0.817	66.5	**36.2**	(30-100)	24
LT	0.210	8.359	0.993	59.7	**20.9**	(40-100)	24
LC	0.230	7.839	0.878	62.1	**22.1**	(40-90)	24
LM	0.297	2.640	0.969	56.5	**19.5**	(20-100)	24
Coffee	0.240	2.467	0.918	165.6	**42.2**	(50-200)	18



TEC_{50}


60
50
40
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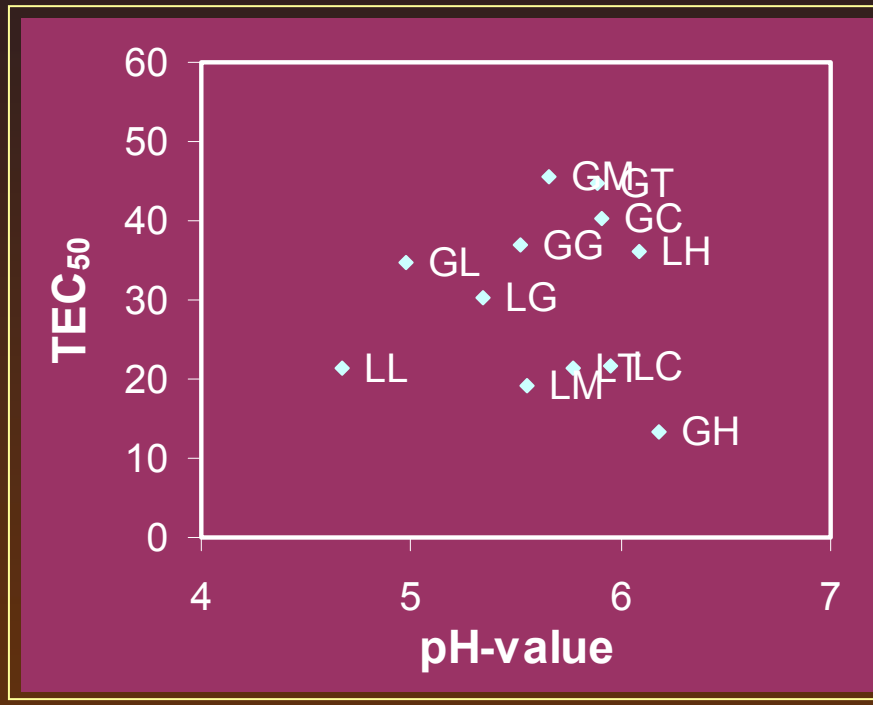
TEC50
GM
GT
GC
GG
LH
GL
LG
LC
LM
LLT
GH
0.00
0.25
0.50
0.75
1.00
A-420


60
50
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10
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TEC50
GM
GT
GC
GG
LH
GL
LG
LL
LM
LTLC
GH
4
5
6
7
pH-value

Antiradical Efficiency

Antiradical Efficiency of Maillard Reaction Mixtures

System	AE	std
GG	**1.7**	0.09
GH	**4.7**	0.23
GL	**1.6**	0.11
GT	**1.9**	0.17
GC	**4.5**	0.09
GM	**1.8**	0.25
LG	**1.8**	0.01
LH	**2.7**	0.29
LL	**1.8**	0.15
LT	**2.9**	0.20
LC	**2.8**	0.17
LM	**2.9**	0.06
Coffee	**3.9**	0.02

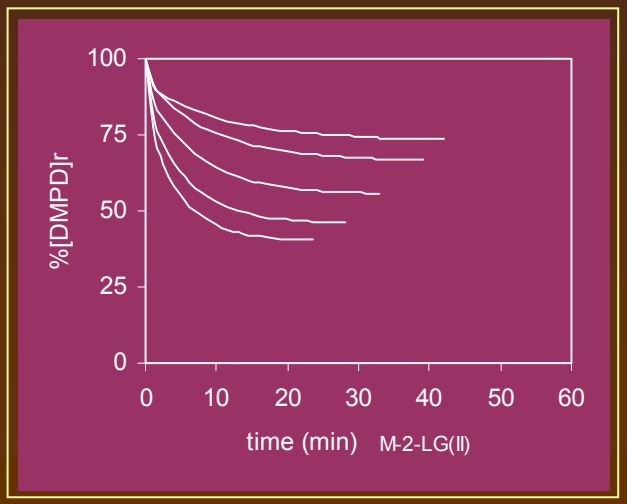
Clasification	System
High	GH / GC
Intermediate	LM / LT / LC / LH
Low	GT / GM / LL / LG / GG / GL

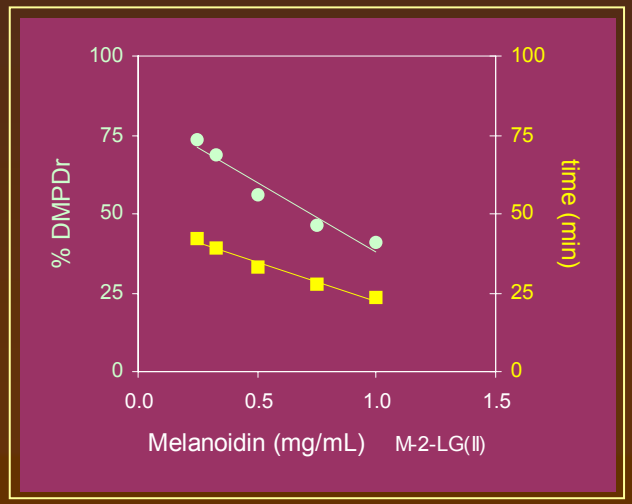
*High (> 4), Intermediate (2 - 4), Low (<2)

Antiradical Eficiency

Limiting conditions:

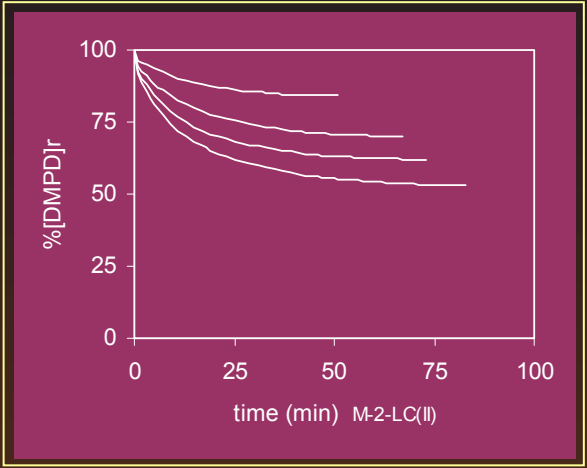
- Low antiradical activity as compared with respective MR mixtures
- Colour of standard melanoidin solution ⇒ < 2 mg/mL
- Some insolubility appeared on LT and GT systems
- Different antiradical behaviour among melanoidins

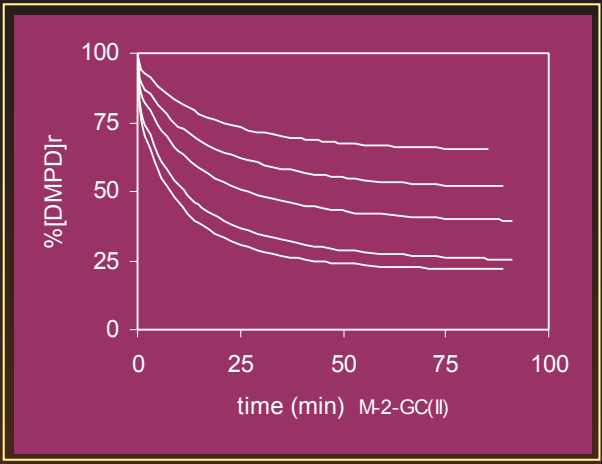


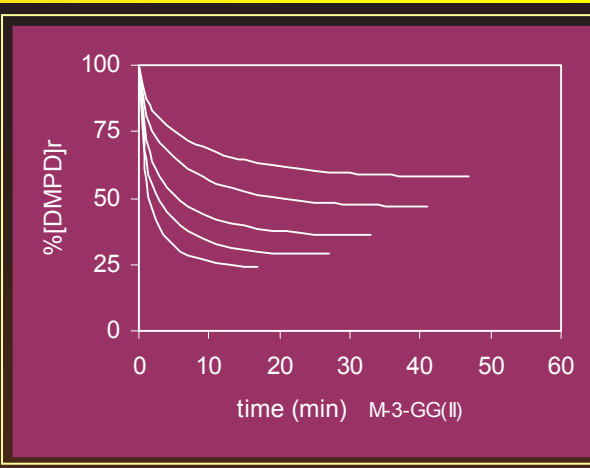


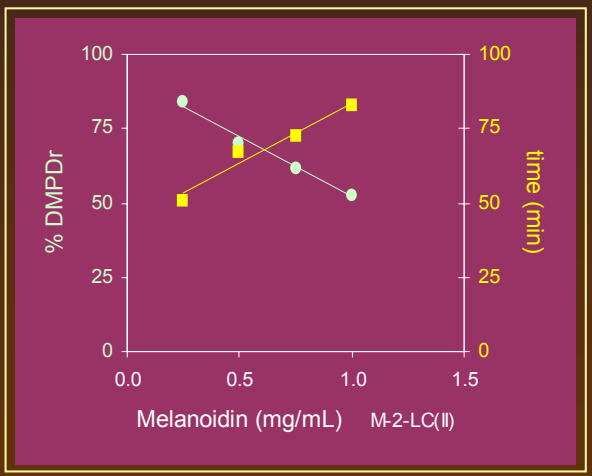
System..... M-LG
range..... 0.25-1.00 mg/mL
EC_{50}..... 0.71 mg/mL
TEC_{50}..... 26.0 min
AE..... 0.0476

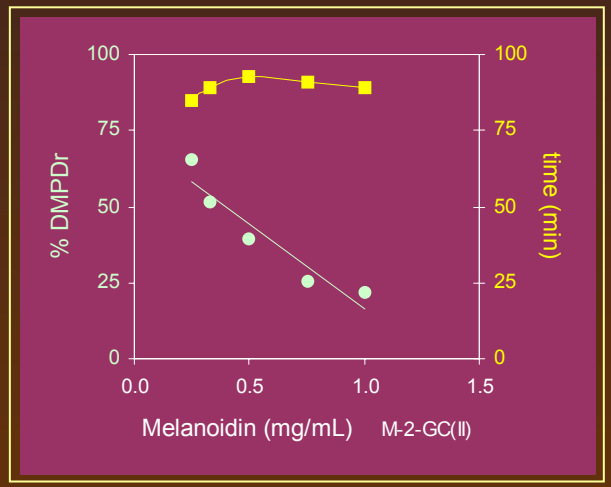
Antiradical Efficiency

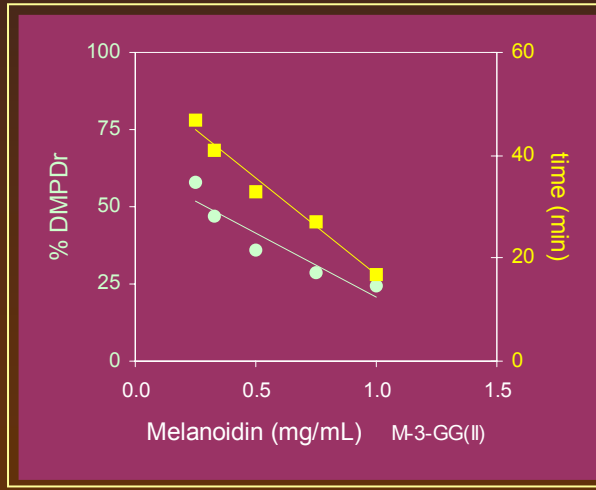










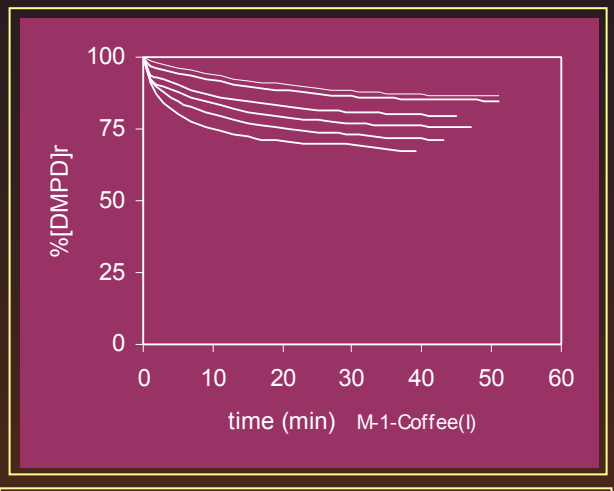


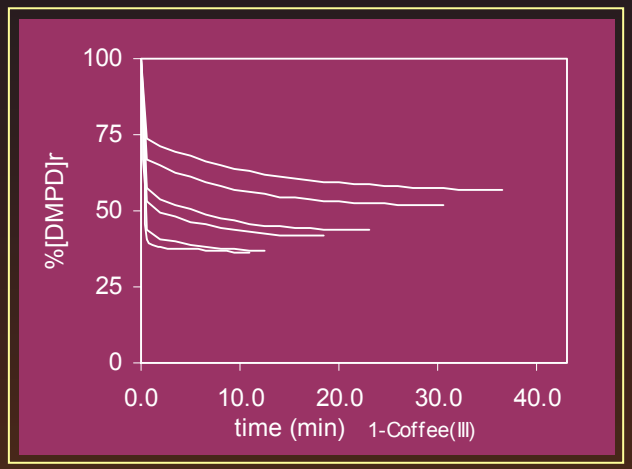
System..... M-LC
range..... 0.25-1.00 mg/mL
EC_{50}..... 1.05 mg/mL
TEC_{50}..... 85.7 min
AE..... 0.0112

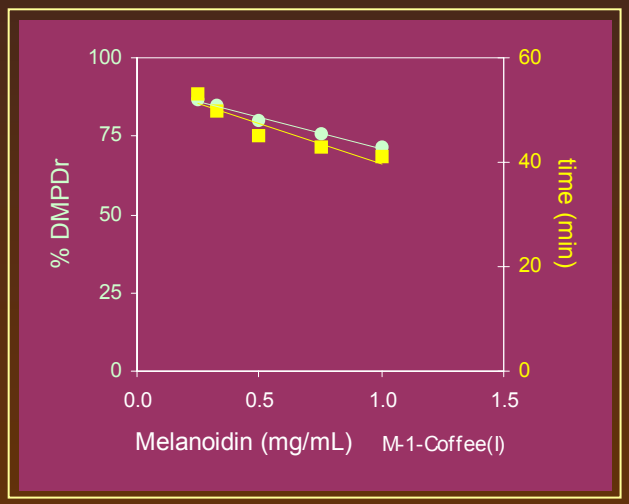
System..... M-GC
range..... 0.25-1.00 mg/mL
EC_{50}..... 0.42 mg/mL
TEC_{50}..... 88.9 min
AE..... 0.0269

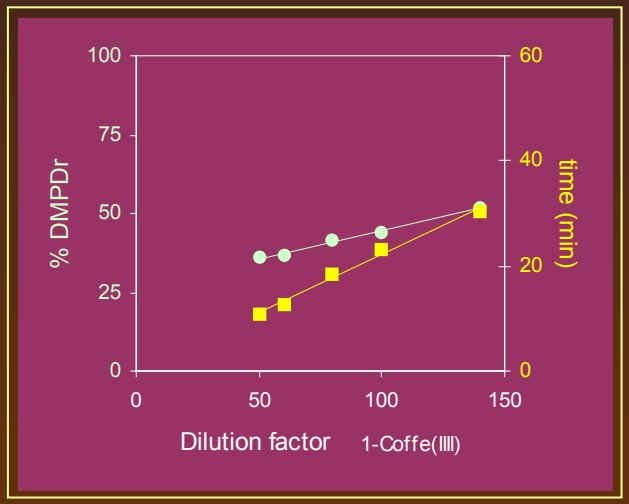
System..... M-GG
range..... 0.25-1.00 mg/mL
EC_{50}..... 0.33 mg/mL
TEC_{50}..... 41.9 min
AE..... 0.0727

Antiradical Efficiency









System..... M-Coffe
range..... 0.25-1.00 mg/mL
EC_{50}..... 2.02 mg/mL
TEC_{50}..... 36.7 min
AE..... 0.0135

System..... Coffee
range..... 50-150 dilution
EC_{50}..... 142.2 dilution
TEC_{50}..... 29.1 min
AE..... 0.0815

Antiradical Efficiency

EC_{50} (mg/mL)

System	EC_{50}	std
GG	0.43	0.162
GC	0.51	0.084
GM	1.11	0.085
GT	1.74	0.065
GL	1.17	0.416
GH	1.50	0.080
LG	0.82	0.140
LC	0.97	0.071
LM	0.96	0.054
LT	1.70	0.119
LL	1.34	0.055
LH	1.11	0.554
Coffee	2.06	0.158

*High (<1), Intermediate (1 - 1.5), Low (1.5 - 2)

Clasification	System
High	GG / GC / LG / LC / LM
Medium	GM / LH / GL / LL / GH
Slow	LT / GT

Standards	EC50
Ascorbic ac.	-----
Gallic ac.	0.0119 mg/mL
Caffeic ac.	0.0144 mg/mL
Ferrulic ac.	0.0186 mg/mL
Trolox	0.0781 mg/mL
Tannic ac.	0.1081 mg/mL

Antiradical Efficiency

TEC_{50} (min)

System	TEC_{50}	std
GG	41.8	19.22
GC	87.8	5.36
GM	51.5	9.50
GT	55.0	7.15
GL	31.0	10.99
GH	18.2	3.71
LG	28.0	5.42
LC	78.5	7.39
LM	21.1	2.22
LT	39.0	13.63
LL	39.0	3.84
LH	10.4	2.55
Coffee	32.2	5.40

Clasification	System
High	LH / GH / LM
Medium	LG / GL / LL / LT / GG
Slow	GM / GT / / / / LC / GC

Standards	TEC50
Gallic ac.	55.7 min
Trolox	38.3 min
Caffeic ac.	34.5 min
Ferrulic ac.	20.1 min
Tannic ac.	13.3 min
Ascorbic ac.	< 1 min

Antiradical Efficiency of Melanoidins

System	AE	std
GG	0.069	0.0133
GC	0.023	0.0028
GM	0.018	0.0020
GT	0.011	0.0010
GL	0.038	0.0194
GH	0.039	0.0086
LG	0.045	0.0026
LC	0.013	0.0015
LM	0.050	0.0043
LT	0.017	0.0060
LL	0.085	0.0368
LH	0.064	0.0225
Coffee	0.015	0.0018

Clasification	System
High	LL / GG / LH
Medium	LM / LG / GH / GL
Slow	GC / GM / GT / LC / GT

Standards	AE
Ferrulic ac.	2.67
Caffeic ac.	2.02
Gallic ac.	1.51
Tannic ac.	0.69
Trolox	0.34
Ascorbic ac.	----

CONCLUSIONS

- DMPD assay is suitable for evaluation of antiradical activity in MR mixtures and melanoidins as well, in watery-fluids.
- It was not observed relationship between browning and EC_{50} or TEC_{50} parameters.
- The AE parameter offers more information about the antiradical activity by taking into account the time of reaction.
- Melanoidins shows lower antiradical activity than MR mixtures.
- MR mixtures did not show important differences of antiradical activity but the EC_{50} could be discriminant for melanoidins according to the type of amino acid.
- Cysteine gave the most powerful antiradical activity (EC_{50}) and histidine gave MR mixtures and melanoidins with shorter time to reach the steady-state (TEC_{50}).
- High molecular weight fraction of coffee (>10kDa) showed lower antiradical capacity than coffee brew solution.