1 **Oxidation stability of muscle with quercetin and rosemary during thermal**

2 **and high-pressure gelation**

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11 **Abstract**

12 The antioxidant activity of quercetin and rosemary extracts were studied in
13 minced fish and in the subsequent treatments to gelation. Both extracts showed
14 antioxidant capacity after processing as it may be deduced from FRAP
15 (reducing capacity) and DPPH (antiradical scavenging) results. The rosemary
16 extract protected more noticeably from lipid oxidation; whereas protein oxidation
17 was prevented by both antioxidants, being quercetin the most efficient
18 antioxidant in those batches subjected to thermal treatment for gel formation
19 (cook and microwave).

20

21

22 **Key words:** antioxidant activity, protein and lipid oxidation, rosemary, quercetin

23

24 **Introduction**

25 There has been an increasing interest in the consumption of restructured
26 minced products in the last years. However, several factors may alter their
27 quality during processing. One of the main factors influencing seafood stability
28 is the oxidation of the different muscle components, due to either the storage
29 conditions or the processing to which minced fish is subjected to obtain and
30 guarantee a safe final product.

31

32 In this sense, a rapid lipid oxidation is frequently observed and a decline in
33 acceptability has been associated with a rise in 2-thiobarbituric acid (TBA)
34 values. However, there are scarcely references about the oxidation of proteins.
35 Formation of carbonyl groups in proteins has been widely used as a measure of
36 oxidation (Srinivasan & Hultin, 1995). Protein oxidation may occur more rapidly
37 than lipid oxidation in biological systems as muscle (Davies & Golberg, 1987;
38 Srinivisan & Hultin, 1995), since protein is within the aqueous phase where
39 many radicals are formed (Soyer & Hultin, 2000). Davies (1986), and Srinivasan
40 and Hultin (2000), described the sequence of changes in proteins as following.
41 Firstly, free radicals react with side chains of proteins, producing protein free
42 radicals. Secondly, these free radicals may react with molecular oxygen to form
43 peroxy radicals, which in turn can capture hydrogen from another molecule
44 yielding hydrogen hydroperoxides. Finally, these protein hydroperoxides may
45 break down, being the carbonyl groups one of the resulting products.
46 Furthermore, protein radicals could react with susceptible lipids enhancing the
47 rate of lipid oxidation, but they could also serve to scavenge free radicals and

48 thus show an antioxidant activity with respect to the lipids (Soyer & Hultin,
49 2000).
50
51 Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated
52 hydroxytoluene (BHT), and α-tocopherol have been used to prevent lipid
53 oxidation in raw and cook mince (Montero, Gómez-Guillén & Borderías, 1996;
54 Weilmeier & Regestein, 2004). However, the test of antioxidants from natural
55 sources for controlling oxidation is receiving considerable attention. Synthetic
56 antioxidants have been widely used in the restructured mince industry (fish and
57 meat), but consumers concern about safety led this industry to find natural
58 sources of antioxidants.
59
60 The application of plant extracts to prevent fish oxidative rancidity has been
61 studied in certain fish products like fillets (Weilmeier & Regestein, 2004;
62 Aubourg, Lugasi, Hovari, Piñeiro, Lebovics, & Jaloczi, 2004), mince gels
63 (Pérez-Mateos, Gómez-Guillén, Hurtado, Solas & Montero, 2002) and
64 emulsions (Frankel, Huang & Aeschbach, 1996). Processing for mince
65 extraction, preparation of batter and mainly cooking, make necessary the
66 addition of antioxidants to avoid the oxidative rancidity. Regarding this subject
67 quercetin and rosemary extracts are some of the most used natural
68 antioxidants.
69
70 Moreover it is important to consider the bio-availability of these antioxidants in
71 the final product. Several *in vitro* techniques are used for this purpose. Two of
72 the most widely used techniques are the measure of the antioxidant activity by

73 FRAP and DPPH• assays. FRAP (t*he ferric reducing/antioxidant power)* assay,
74 measures the reducing ability of the antioxidant (Benzie & Strain, 1996);
75 whereas DPPH• determination evaluates the antioxidant ability to scanvege a
76 free synthetic radical (2,2-diphenyl-1-picryhydrazyl DPPH•) (Brand-Williams,
77 Cuvelier & Berset, 1995; Sánchez-Moreno, Larrauri & Saura-Calixto, 1998).
78
79 This study investigated the *in vitro* bio-availability and the effects of quercetin
80 and rosemary extracts to prevent lipid and protein oxidation in mince fish
81 muscle, as well as in subsequent treatments such as homogenization to form a
82 batter and gelation induced by both, heat (traditional and microwave) and high
83 pressure.
84
85 **Material and methods**
86
87 **Samples preparation**.
88 Atlantic mackerel (*Scomber scombrus*) used in this study were caught at the
89 Cantabrian coast and kept at 4 ºC for 48 h. Fish (14 Kg) were headed, gutted
90 and washed. Skin and bones were removed with a deboning machine with a
91 pore of 3 mm (Baader 694, Lübeck, Germany).
92
93 Proximate analyses of mince were performed according to Association of
94 Official Analytical Chemists procedures (1989): moisture (method 24003), ash
95 (method 1821), and protein (method 24024). Crude fat was determined
96 following the method of Bligh and Dyer (1959). Proximate analyses were: total

97 protein 17.95 % ± 0.53, moisture 76.85 % ± 0.16, total fat 3.78 % ± 0.09, and
98 ash 0.96 % ± 0.01.
99
100 The mince was divided in three batches: minced muscle (M), mince muscle plus
101 0.3 % of quercetin extract (MQ) and mince muscle plus 0.6 % of rosemary
102 extract (MR). The natural antioxidants quercetin and rosemary extract
103 (Altaquímica. Barcelona, Spain) were added in these proportions to get
104 antioxidant activity according to preliminary trials. Both ingredients were
105 blended up to a homogeneous distribution.
106
107 The batch consisted of minced muscle was divided in four sub-batches. Three
108 of them were placed in a refrigerated vacuum homogenizer (Stephan UM5,
109 Stephan u. Söhne GmbH & Co., Germany), and ground for 1 min at high speed.
110 Sodium chloride (2 % w/w) (Panreac, Montplet & Esteban S.A., Barcelona,
111 Spain) was added and homogenized for 3 min at slow speed. Quercetin extract
112 (0.3 %) was added to the first sub-batch (*sol* Q) with crushed ice to give the
113 required final moisture (77 %), whereas rosemary extract (0.6%) was added to
114 the second one (*sol* R). No antioxidant extract was added to the third sub-batch
115 (sol). The homogenate was beaten slowly for 5 min under vacuum, with the
116 temperature being maintained below 10 ºC.
117
118 Protein, fat and moisture of sol and gel formulation were calculated from the
119 proximate analyses carried out on the mince. Mince plus sodium chloride (*sol*
120 and gel formulation) were 77.54 % water, 16.55 % protein and 3.39 % fat; *sol*
121 and gel formulation with quercetin were 77.27 % water, 16.25 % protein and

122 3.31 % fat; and *sol* and gel formulation with rosemary were 77.28 % water,

123 16.00 % protein and 3.25 % fat.

124

125 **Gel forming treatments**.

126 Homogenates with sodium chloride were stuffed into flexible plastic casing

127 (Krehalon Soplaril, Barcelona, Spain) of 40 µm thickness and 3.5 cm diameter.

128 Conventional thermal treatment was used for gel formation (90 ºC / 50 min)

129 (TQ; TR). High pressure treatments were performed in a high pressure pilot unit

130 (ACB 665, Gec Alsthom, Nantes, France), where the temperature of immersion

131 medium (distilled water) was controlled via a thermocouple with a programmed

132 thermostatization equipment (model IA/2230 AC, INMASA, Barcelona, Spain).

133 The pressure was increased by 2,5 MPa/s. The high pressure treatments

134 applied were 300 MPa / 25 °C / 15 min (P25Q, P25R) and 300 MPa / 5-7 °C /

135 15 min (P7Q, P7R). A microwave treatment was also tested to obtain the gels,

136 consisted of 700 W during 90 sec (45 sec on one side and it was turn over 45

137 sec) (mwQ, mwR); where water was used to cover the bottom of plate.

138

139 **Antioxidant activity**

140 The ferric reducing/antioxidant power (FRAP) assay was used as a measure of

141 the reducing ability of gels following the method Benzie and Strain (1996). It is

142 based on the increase in absorbance at 595 nm due to the formation of the

143 complex tripiridiltriazine (TPTZ)-Fe(II) in the presence of tissue reducing agents.

144 Absorbance was read at 4 and 30 min. The parameter Equivalent concentration

145 1 or EC_1 was defined as the concentration of antioxidant having a ferric-TPTZ

146 reducing ability equivalent to that of 1 mmol/L $FeSO_4.7H_2O$ EC_1 was calculated

147 as the concentration of antioxidant giving an absorbance increase in the FRAP
148 assay equivalent to the theoretical absorbance value of a 1 mmol/L
149 concentration of Fe(II) solution, determined using the corresponding regression
150 equation.
151

152 **Free Radical Scavenging Measurement**.
153 The anti-radical capacity of the sample extracts and pure compounds (quercetin
154 and rosemary), was estimated according to the procedure reported by Brand-
155 Williams et al. (1995), slightly modified by Sánchez-Moreno et al. (1998).
156 Samples were thawed and the extracts were obtained by homogenizing 10 g of
157 each one with 50 mL of methanol (Omni-mixer, Type OM, Ivan Sorvall, Inc,
158 Norwalk Conn., USA) during 2 min (setting 6) in a bath containing water and
159 ice. Afterwards, the extracts were filtered under vacuum. The data is reported
160 as EC_{50}, which is the concentration of antioxidant required for 50 % scavenging
161 of DPPH• radical. The specified time (T_{EC50}) is the time needed to reach a
162 steady state at the concentration corresponding to EC_{50}.
163

164 **Protein oxidation**.
165 Determination of *carbonil radical* was performed following the method described
166 by Srinivisan and Hultin, 1995. Results were expressed as nmol per mg protein.
167

168 **Lipid oxidation**
169 *TBA index* (thiobarbituric acid) was determined following the method of Vyncke
170 (1970), incubating at 90 ºC for 40 min. Results were expressed as µmol
171 malonaldehyde per 100 g of sample (muscle or gel, respectively).

172

173 **Statistical analysis**

174 Two-way analysis of variance was run. The computer program used was the
175 Statgraphics Plus (Rockville, MD, USA) statistical program. Pairwise
176 comparison of the differences between means was performed using Duncan's
177 test with confidence intervals set for a level of significance of $p \leq 0.05$.

178

179 **Results and discussion**

180 Preliminary trials showed that it was necessary to use rosemary extract in
181 double concentration than quercetin in order to obtain a similar range of
182 antioxidant activity. In the present study the antioxidant capacity of different
183 batches of minced muscle, *sol* and gels containing quercetin (0.3 %) and
184 rosemary (0.6 %) extracts, measured by ferric reducing/antioxidant power assay
185 (FRAP), is shown in Figure 1. It was noticeable the high FRAP values
186 corresponding to the samples which contained quercetin, compared to those
187 including rosemary, despite the latter being in double concentration. Mince
188 muscle (M) and mince muscle homogenized with NaCl (2 %) to form a batter
189 (*sol*) did not show any ability as reductants. However, both muscle and *sol*
190 containing either quercetin or rosemary showed this ability, being considerably
191 higher ($p \leq 0.05$) in those samples with quercetin. The *sol* with quercetin (*sol* Q)
192 sample showed a significantly lower activity than muscle with quercetin (MQ),
193 when FRAP index was measured after 30 min reaction. This was probably due
194 to the prooxidant effect of NaCl solubilized in the muscle, as it has been
195 previously described (Andersen & Skibsted, 1991; Kanner, Harrel & Jafe, 1991).
196 No decrease was found in the reducing ability when *sol* with antioxidants was

197 subjected to gelation by conventional heat treatment. Furthermore, a slight but
198 significant increase was found in FRAP values ($p \leq 0.05$) corresponding to gels
199 induced by high pressure at 7 ºC and, specially, at 25 ºC, containing both
200 quercetin and rosemary. It seems that high pressure as treatment for gel
201 induction may promote the antioxidant activity. This could be due to either a
202 direct or indirect effect on antioxidants (rosemary and quercetin). High pressure
203 may lead to reduce the interactions between these antioxidants and muscle
204 compounds, giving a higher availability of the antioxidant molecules to hinder
205 oxidation phenomena. Furthermore, it is known that high pressure itself has a
206 lipid prooxidant effect, which may be diminished by the addition of certain
207 antioxidants like rosemary extract (Pérez-Mateos et al., 2002). Microwave
208 treatments led to similar FRAP values to those found in case of batters and gels
209 obtained by conventional heat treatment for both antioxidants. The pure
210 quercetin extract at 0.3 % showed a similar reducing ability to that described for
211 MQ, mean while pure rosemary extract at 0.6 % showed about 6 times higher
212 ability than M R. The loss of activity observed for rosemary extract in muscle
213 and gel could be due to its role in lipid oxidation, which consequently involves a
214 reduction in the bio-availability of this antioxidant. It has been reported that
215 rosemary extract reduces the lipid oxidation in gels induced by heat and high
216 pressure (Pérez-Mateos et al., 2002).

217

218 It was observed that the reducing capacity was not stable with reaction time, but
219 tended to increase from measure at 4 min up to 30 min in all the batches. This
220 increase was about 20 % in samples including rosemary and 35 % - 40 % in
221 those containing quercetin. These results are in accordance with data reported

222 by Benzie and Strain (1996) for pure extract of phenolic compounds.
223 Considering these results, the increase observed in the reducing ability with
224 reaction time could be considered another parameter to define the antioxidant
225 capacity of a compound**.**

226

227 The antioxidant activity measured by the scavenging of the synthetic radical
228 (2,2-diphenyl-1-picryhydrazyl DPPH•) is shown in table 1. EC_{50} is the
229 concentration of antioxidant required for 50 % scavenging of DPPH• radical in a
230 period of time T_{EC50}. Short times and low concentrations are important to define
231 a good antioxidant activity. For quercetin and rosemary pure extracts EC_{50} was
232 0.12 mg/mL and 0.9 mg/mL, whereas T_{EC50} was 4.5 min and 7.5 min,
233 respectively, indicating the higher *in vitro* antioxidant effect of quercetin extract.
234 Regarding this subject, it could be considered that quercetin and rosemary
235 extracts show a medium standard antioxidant activity, given that concentration
236 is quite low but time is not according with Sánchez-Moreno et al. (1998). These
237 authors classified the kinetic behaviour of different antioxidant compounds
238 according to T_{EC50} values. Ascorbic acid was an example of rapid kinetic
239 behaviour since the time needed to achieve a steady state (T_{EC50}) was less than
240 5 minute. α-Tocopherol was classified as intermediate, with a T_{EC50} value within
241 the interval 5 - 30 minutes, and rutin was an example of slow kinetic behaviour
242 with higher T_{EC50} values.

243

244 When quercetin and rosemary extracts were blended with muscle (MQ and M
245 R), they lost efficiency and the EC_{50} noticeably increased, being higher in case
246 of MR. However, T_{EC50} values were similar for both, MQ and MR. The addition

247 of salt did not involve any change in EC_{50}, and *sol* R and *sol* Q showed similar
248 values to those described for MR and MQ, respectively. Nevertheless, *sol* Q
249 showed quite higher T_{EC50} values than MQ. The lost of antioxidant activity
250 observed with respect to pure extracts was probably due to the interaction
251 between antioxidant molecules and mince or *sol* constituents, respectively. The
252 antioxidant capacity in gels induced by conventional heat treatment was similar
253 to that found for both, mince muscle enriched with antioxidants and gels
254 induced by high pressure applied at moderate temperature (25 ºC). However,
255 the gels induced by high pressure in cold conditions (7 ºC) containing quercetin
256 showed increased EC_{50} values and thus, lower scavenging antioxidant capacity.
257 On the other hand, those gels obtained by microwave treatment showed the
258 best antioxidant capacity, which resulted similar to that found for pure rosemary
259 and quercetin extracts. It seems that the short time needed to obtain the
260 microwave gels promotes a rapid protein-protein interaction and, thus, is not
261 enough to establish any bond between muscle protein and antioxidant
262 molecules, remaining the latter more bio-available.
263
264 Although T_{EC50} values were lower in pure quercetin extract than in rosemary
265 extract, the opposite behaviour was observed when these antioxidants were
266 added to mince, *sol* or gel, finding the lowest T_{EC50} values in samples including
267 rosemary. Nevertheless, according to the antioxidant kinetic classification
268 defined by Sánchez-Moreno et al. (1998), an intermediate kinetic behaviour was
269 found in most cases.
270

271 The mechanism of protection given by an antioxidant was postulated to occur at
272 the initial stage and more effectively during the propagation stage of oxidation in
273 case of peroxy radical (ROO▪) scavengers such as phenolic compounds. The
274 peroxy radicals formed are intercepted or inhibited by a free radical acceptor
275 (phenolic structure), stopping the chain reaction as a consequence (Basaga,
276 Tekkaya & Acikel, 1997).
277
278 Since the antioxidants used in the present study are chiefly phenol based
279 compounds, the determination of radical scavenging capacity should be an
280 adequate method to evaluate the antioxidant ability of the pure rosemary and
281 quercetin extracts. However, it is possible that some interactions with lipids and
282 proteins take place when these antioxidants are blended with mince muscle
283 and, as a result of this, they partially loose its scavenger capacity.
284
285 The reducing ability of these polyphenols (quercetin and rosemary) seems to be
286 a more important factor to dictate the antioxidant activity than the free-radical-
287 scavenging capacity, given that differences were less noticeable in the latter.
288 Each method mentioned above measures the ability of the antioxidants in
289 different steps of the oxidative chain, and thus the mechanism of antioxidative
290 action is different. The ability of monomeric phenolic compounds as antioxidants
291 depends on both, the degree of hydroxylation and the extent of conjugation
292 (Hodnick, Milosevljevic, Nelson & Pardini, 1988). However, there are not studies
293 to evaluate the remaining antioxidant activity in a substrate like mince fish and
294 the products obtained after gelation. Regarding this subject, it is necessary to
295 take into account possible interactions between antioxidant molecules and

296 mince compounds that influence the bio-availability of the antioxidants.
297 Quercetin extract seems to be more bio-available than rosemary extract when
298 included into a fish gel matrix.
299

300 The carbonyl groups content, measured as an index of protein oxidation, is
301 shown in figure 2. The addition of quercetin to mince muscle slightly increased
302 ($p \leq 0.05$) the carbonyl groups content. This result seems to be in conflict with
303 the antioxidant properties of the quercetin extract. It could be possible that, as a
304 consequence of the interactions that may take place between quercetin and
305 protein, new susceptible to oxidation sites arose in proteins. The solubilization
306 of protein with sodium chloride gave rise to a noticeable increase in carbonyl
307 groups level, despite having a lower content of protein (1.5 %). This effect has
308 been previously reported in other studies (Karastogiannidou, 1999). The native
309 structure of the protein is often the most stable conformation, and a chemical
310 change in the side groups may probably lead to a partial loss of stability (Hultin,
311 1986). Both extracts, quercetin and especially rosemary, acted as antioxidants
312 and decreased the carbonyl groups content in *sol* samples. No differences in
313 carbonyl groups content were found between gels induced by conventional heat
314 or microwave treatment, respectively. However, the presence of quercetin
315 extract gave rise to noticeable lower levels of carbonyl groups. Furthermore, it
316 seems that high pressure treatment promoted the formation of carbonyl groups
317 in spite of the addition of rosemary or quercetin extract, although levels were
318 higher in those gels including rosemary, mainly when high pressure was applied
319 at moderate temperature (25 ºC). The high pressure induced protein oxidation
320 more than thermal treatments. Quercetin extract seems to be more effective

321 when the gel networks are formed by thermal treatments rather than by high
322 pressure treatment.
323
324 Formation of thiobarbituric acid reactive substances (TBARS) is shown in figure
325 3. The addition of antioxidant extracts to mince muscle did not substantially
326 modify the lipid oxidation level, probably because a slight blending did not
327 induce oxidation.
328
329 However, the homogenization of minced muscle with salt caused a two-fold
330 increase in lipid oxidation ($p \leq 0.05$), despite the fat content in M was slightly
331 lower than in *sol*. It is known that sodium chloride has a prooxidant effect,
332 speeding up the formation of TBARS (Karastogiannidou, 1999). The inclusion of
333 antioxidants in the formula led to a decrease in TBARS values ($p \leq 0.05$),
334 especially in case of rosemary. Conventional thermal treatment significantly
335 decreased the oxidation level found in *sol* and mince samples ($p \leq 0.05$),
336 probably because there was a slight variation in formula, mainly in protein
337 content (2.17 % lower). In addition, lipids may interact covalently with proteins
338 upon gelling, leading to a considerable reduction of the amount of available TBA
339 reactive substances. The high pressure gave rise to similar values at 7 ºC and
340 25 ºC respectively, for rosemary and quercetin, although rosemary was shown
341 more effective than quercetin extract. TBARS values for gels induced by
342 microwave treatment including both, quercetin and rosemary, were similar to
343 those described for gels induced by high pressure containing rosemary.
344

345 In general, the effectiveness of rosemary to prevent lipid oxidation was higher
346 than that shown by quercetin although it should be taken into account that the
347 former was added in double amount. Rosemary gave rise to a lower antioxidant
348 activity measured by FRAP when added to mince muscle, *sol* and gels,
349 although the pure rosemary extract presented higher FRAP values than pure
350 quercetin. Thus, it seems that rosemary may interact with lipids in a higher
351 degree, preventing them from oxidation. Quercetin and rosemary extracts
352 remained partially bio-available in the final gels. Rosemary gave rise to a higher
353 protection against lipid oxidation in gels induced by heat and high pressure
354 treatment, meanwhile quercetin seemed to be the most effective against
355 oxidation of proteins, mainly in gels induced by conventional heat treatment.
356

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359

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449

450 **Legend of figures**

451

452 **Figure 1**. Total antioxidant activity by FRAP of different samples: mince (M),
453 mince with antioxidant (MQ and MR), batter or *sol*, *sol* with antioxidant (*sol* Q
454 and *sol* R), gel induced at cook temperature 90 ºC (TQ and TR), gel induced at
455 300 MPa of high pressure/ 7 ºC (P7Q and P7 R), gel induced at 300 MPa of
456 high pressure/ 25 ºC (P25Q and P25 R) and gel induced by microwave (mwQ
457 and mwR). Different letters (a, b, c,..) indicate significant differences ($p \leq 0.05$)
458 among samples, different letters (z, y, x) indicate significant differences ($p \leq$
459 0.05) between times of measurement for the same sample.

460

461 **Figure 2**. Carbonyl groups content (mol / gr protein) of different samples: mince
462 (M), mince with antioxidant (MQ and MR), batter or *sol*, *sol* with antioxidants
463 (*sol* Q and *sol* R), gel induced at cook temperature 90 ºC (TQ and TR), gel
464 induced at 300 MPa of high pressure/ 7 ºC (P7 Q and P7R), gel induced at 300
465 MPa of high pressure/ 25 ºC (P25 Q and P25R) and gel induced by microwave
466 (mw Q and mwR). Different letters (a, b, c,..) indicate significant differences ($p \leq$
467 0.05) among samples.

468

469 **Figure 3**. Thiobarbituric acid (TBA) index of different samples: mince (M), mince
470 with antioxidant (MQ and MR), batter or *sol*, *sol* with antioxidant (*sol* Q and *sol*
471 R), gel induced at cook temperature 90 ºC (TQ and TR), gel induced at 300
472 MPa of high pressure/ 7 ºC (P7 Q and P7R), gel induced at 300 MPa of high
473 pressure/ 25ºC (P25 Q and P25R) and gel induced by microwave (mw Q and

474 mwR). Different letters (a, b, c,..) indicate significant differences ($p \leq 0.05$)

475 among samples.

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478 **Table 1**. Total antioxidant activity by DPPH• of different samples mince (M),
479 mince with antioxidant (MQ and MR), batter or *sol*, *sol* with antioxidant (*sol* Q
480 and *sol* R), gel induced at cook temperature 90ºC (TQ and TR), gel induced at
481 300 MPa of high pressure/ 7ºC (P7 Q and P7R), gel induced at 300 MPa of high
482 pressure/ 25ºC (P25 Q and P25R) and gel induced by microwave (µw Q and
483 µwR).

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	EC_{50} (mg/mL)	**T_{EC50} (min)**
M	nd	
M Q	0.24	5.0
MR	1.2	3.8
Sol	nd	
Sol Q	0.2	22
Sol R	1.05	3.3
TQ	0.26	3.6
TR	1.8	2.9
P7 Q	0.7	6.6
P7R	1.4	1.8
P25 Q	0.29	3.3
P25R	1.6	2.8
mwave Q	0.15	9.0
mwaveR	0.6	6.25

486 nd: not detectable

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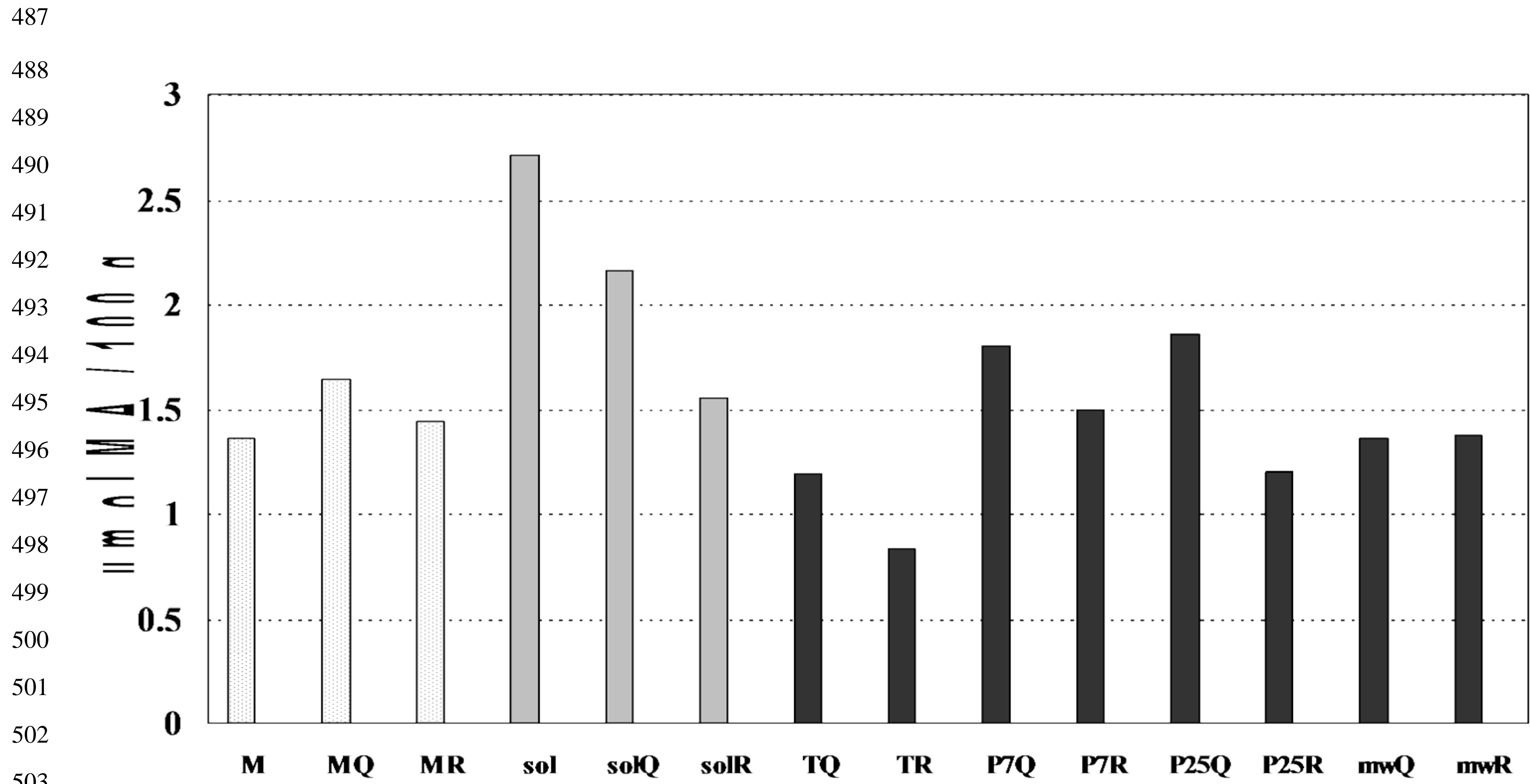

504 **Figure 3**

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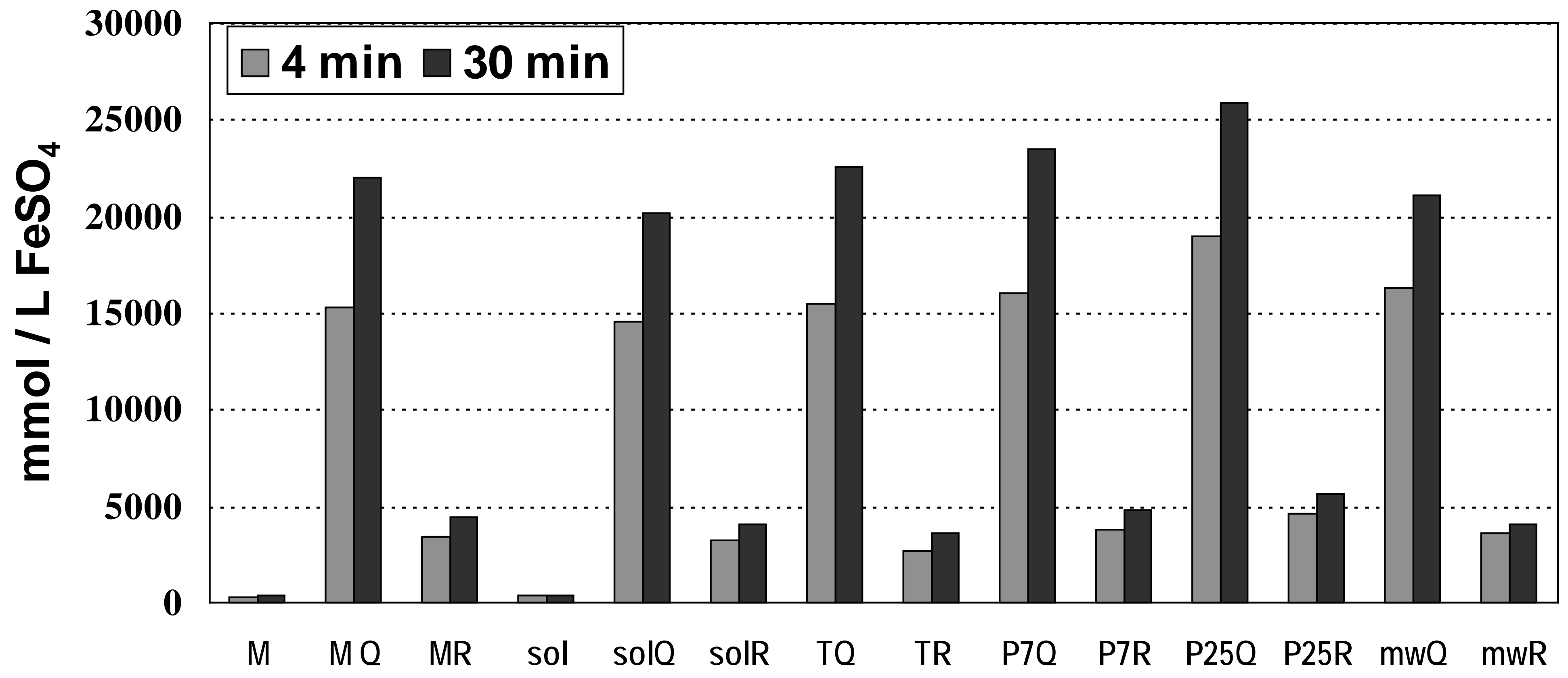

522 **Figure 1**

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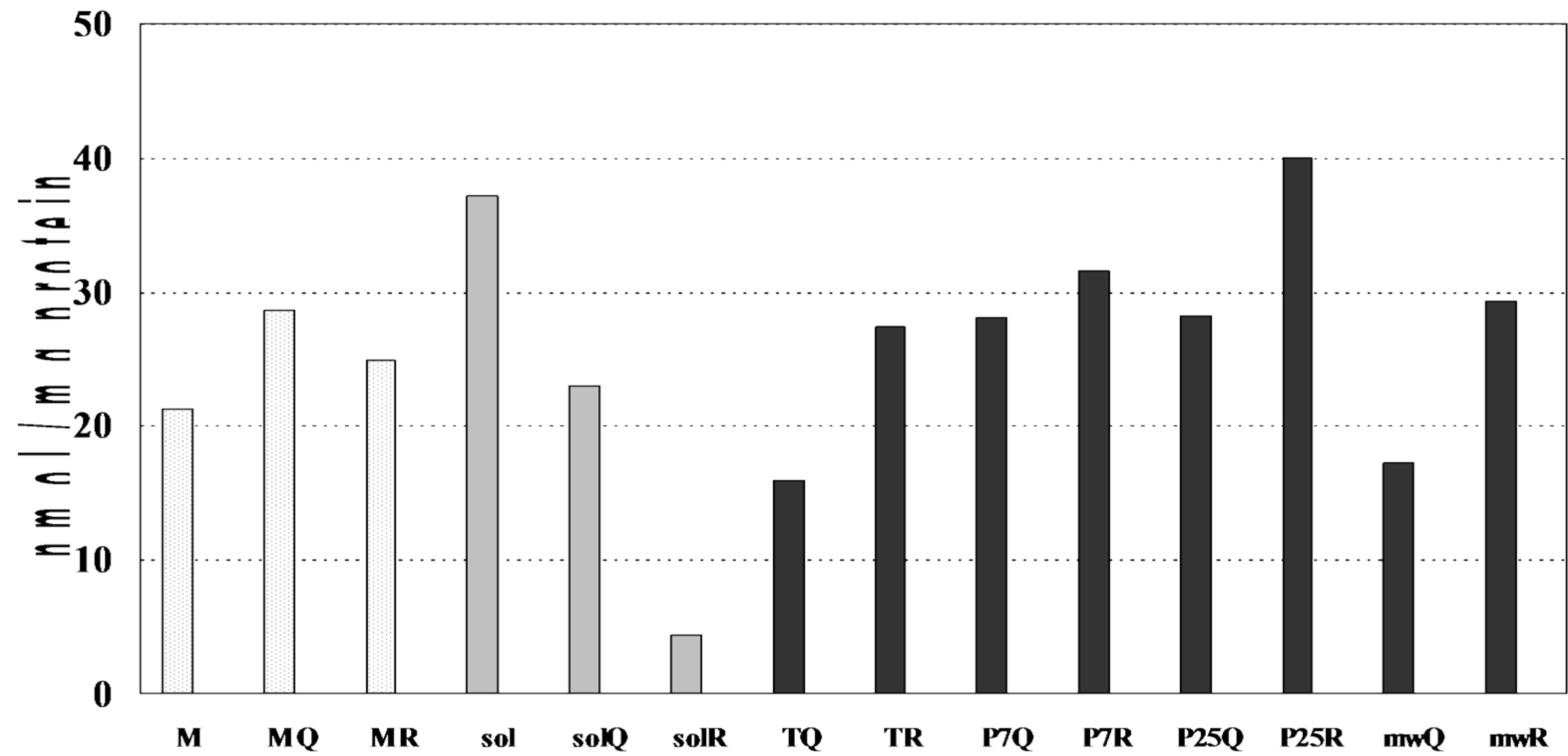

540 Figure 2

Food Chem. 2011;124(4):1393-1403.