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1 Effect of oxidation on the gel properties of porcine myofibrillar proteins and their
2 binding abilities with selected flavour compounds

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17 **Abstract:**

18 In this work, the effect of oxidation induced by hydroxyl radicals on the binding
19 abilities of myofibrillar protein (MP) gels to aldehydes and ketones and their
20 relationship with MP gel properties were investigated. Mild oxidation (0–0.2 mM H₂O₂)
21 could induce partial unfolding of MP, thus slightly increasing the salt solubility of MP
22 and enhancing the hardness of MP gels. MP suffering a higher oxidative attack could
23 undergo a reduction in water-holding capacity, with increased mobility of water in MP
24 gels. Oxidation could make MP gel more disordered. The ability of oxidised MP gels
25 to bind to flavours decreased as the carbon chain length of the flavour compound
26 increased. MP oxidation only significantly affected the binding of MP gels to hexanal,
27 heptanal, and 2-octanone, while other flavour compounds were not affected.

28 **Keywords:** myofibrillar proteins; oxidation; gel properties; flavours release; water
29 mobility

30 **1. Introduction**

31 The formation of protein gels in processed muscle foods is an important functionality
32 that can affect the texture and sensory characteristics of the final meat products.
33 Myofibrillar proteins (MP), as the major proteins in muscle, are excellent gelling agents
34 that are largely responsible for the textural and structural characteristics of meat
35 products (Xiong, Blanchard, Ooizumi, & Ma, 2010).

36 During meat storage or industrial processing, reactive oxygen species, which include
37 superoxide anions, hydroxyl radicals, peroxy radicals and lipid oxidation products,
38 play a critical role in the accumulation of oxidative damage in proteins (Zhou, Zhao,
39 Zhao, Sun, & Cui, 2014a). Types of oxidative damage to muscle proteins can include
40 conformational changes, peptide chain scission and formation of amino acid derivatives
41 or aggregates, leading to changes in physicochemical properties of proteins, which in
42 turn alter the functional properties of proteins, such as gelation, emulsification and the
43 capacity to bind flavours (Sun, Zhou, Sun, & Zhao, 2013; Xiong et al., 2010). The gel
44 properties of MP, such as rheological properties, water-holding capacity (WHC),
45 texture and microstructure change under oxidative stress (Xiong, Park, & Ooizumi,
46 2009).

47 Food acceptability by consumers is governed to a considerable extent by their
48 organoleptic properties, and mostly by flavour perception (Gierczynski, Guichard, &
49 Laboure, 2011). Volatile flavour release is largely determined by the tendency of
50 volatile compounds to bind to other ingredients (particularly oils and protein) and by
51 food microstructure (Guichard, 2002). As mentioned above, protein oxidation can
52 affect meat products microstructure, especially gel quality. Some reports have
53 evaluated the abilities of oxidised MP at different oxidation levels to bind to flavour
54 compounds (Cao, Zhou, Wang, Sun, & Pan, 2018; Zhou et al., 2014a). Thus far,

55 investigations regarding the binding of oxidised MP gels to flavour compounds are
56 limited. Modifying food microstructure can also control volatile release in foods (Mao,
57 Roos, & Miao, 2014). Among flavours, the impacts of aldehydes and ketones are of
58 particular interest because of their practical contribution in meat and meat products
59 (Guichard, 2002). Therefore, it is necessary to investigate the behaviour of oxidised MP
60 gels in binding to typical odour-active aldehydes and ketones, to establish the
61 mechanism of flavour release in gelatinous meat products.

62 In this work, MP were exposed to a hydroxyl radical-generating system that is
63 commonly involved in meat and meat products. Examination of the accompanying
64 protein structural changes (sulfhydryl [SH] groups, surface hydrophobicity, salt
65 solubility, particle size distribution) with the results of sodium dodecyl sulfate-
66 polyacrylamide gel electrophoresis [SDS-PAGE]) and with gel properties (hardness,
67 WHC, microstructure, low-field nuclear magnetic resonance [LF-NMR] relaxation
68 time) was designed to investigate the influence of oxidative modification on MP and
69 MP gels. The phenomena of oxidised MP gels binding to typical aldehyde and ketone
70 compounds were evaluated using headspace analysis followed by gas chromatography-
71 mass spectrometry. Moreover, selected aldehyde and ketone compounds of various
72 chain lengths were used to evaluate the contributions of molecular structure to the
73 binding phenomena. The relationship between MP gel properties and their binding
74 abilities is discussed.

75 **2. Materials and methods**

76 *2.1. Materials*

77 Fresh porcine muscle (*longissimus dorsi*) was purchased from a local commercial
78 abattoir (Guangzhou, China), where the pigs were slaughtered at approximately 6
79 months of age following standard industrial procedures. Fat and connective tissue were

80 removed before the separation of proteins. Pentanal, hexanal, heptanal, octanal, 2-
81 pentanone, 2-heptanone, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and piperazine-
82 *N,N*-bis(2-ethanesulfonic acid) (PIPES) were purchased from Sigma-Aldrich Chemical
83 Co. (St. Louis, MO); 2-hexanone, 2-octanone, propyl gallate and Trolox C were
84 obtained from Aladdin (Shanghai, China). Bromophenol blue (BPB), thiourea,
85 dithiothreitol and EDTA were obtained from Sinopharm Chemical Reagent Co., Ltd.
86 (Shanghai, China). All other chemicals were of analytical reagent grade at minimum.

87 *2.2. Preparation of MP*

88 MP were extracted according to the method of Shen, Zhao and Sun (2019), with a
89 slight modification. The pH of the MP suspension (0.1 M NaCl) in the last wash was
90 adjusted to 6.25 before centrifugation (2000 g, 15 min, 4 °C). The concentrations of MP
91 were measured by the Biuret method using bovine serum albumin as a standard. The
92 MP pellet was kept on ice and used within 2 days.

93 *2.3. Protein oxidation*

94 MP were suspended (30 mg/mL) in a 15-mM PIPES buffer containing 0.6 M NaCl
95 (pH 6.25). To oxidise them, MP suspensions were incubated at 4 °C for 24 h, using a
96 hydroxyl radical-generating system. The hydroxyl radicals were produced by a 10- μ M
97 FeCl₃/100- μ M ascorbic acid solution with 1 mM H₂O₂. Oxidation was terminated by
98 adding propyl gallate/Trolox C/EDTA (1 mM each) (Xiong et al., 2009). The fresh MP
99 suspension (30 mg/mL) without any hydroxyl radical-generating system or terminating
100 agent was used as the control. The concentrations of MP in the following measurements
101 were adjusted using a 15 mM PIPES buffer (pH 6.25) containing 0.6 M NaCl.

102 *2.4. Total and reactive SH groups*

103 Total SH contents were determined with a DTNB method with some modifications
104 (Zhou, Zhao, Su, & Sun, 2014b). For determining the level of total SH groups, 1 mL

105 MP suspension (4 mg/mL) was mixed with 5 mL of 0.086 M Tris-Gly buffer (5 mM
106 EDTA, 8 M urea, 0.6 M NaCl, pH 8.0) and 30 μ L of 4 mg/mL DTNB (0.086 M Tris-
107 Gly buffer, 5 mM EDTA, 0.6 NaCl, pH 8.0). After incubation at room temperature (25
108 \pm 1 $^{\circ}$ C) for 30 min, the absorbance at 412 nm was recorded for the calculation of total
109 SH groups using a molar extinction coefficient of 13,600 M⁻¹ cm⁻¹. Reactive SH groups
110 were prepared by incubating the reaction mixture in the absence of urea. The blank was
111 run with 15-mM PIPES buffer (pH 6.25) containing 0.6-M NaCl.

112 2.5. *Surface hydrophobicity*

113 The surface hydrophobicity of the MP was measured by the hydrophobic
114 chromophore BPB method (Chelh, Gatellier, & Santé-Lhoutellier, 2006) with a slight
115 modification. This method can determine the surface hydrophobicity of MP, avoiding
116 the solubilisation step of the myofibrils before the protein hydrophobicity determination.
117 The MP suspension (1 mL, 4 mg/mL) was thoroughly mixed with 100 μ L BPB (1
118 mg/mL) and kept at ambient temperature (25 \pm 1 $^{\circ}$ C) for 10 min before centrifugation
119 (5000 g, 15 min, 25 $^{\circ}$ C). The absorbance of each supernatant (diluted for 10 times) was
120 determined at 595 nm against a PIPES buffer blank. A sample with the same treatments
121 in the absence of MP was used as the control. The index of surface hydrophobicity was
122 expressed as the amount of bound BPB, and it was calculated using the following
123 formula (1):

$$124 \quad \text{BPB bound } (\mu\text{g}) = 100 \times (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \quad (1)$$

125 2.6. *Salt solubility and MP turbidity*

126 Salt solubility of MP (10 mg/mL) and turbidity of MP suspensions (1 mg/mL) were
127 determined according to Shen et al. (2019). The salt solubility of MPs was measured
128 after centrifuging (5000 g, 25 $^{\circ}$ C) for 20 min to separate the salt-soluble fractions from

129 the insoluble fractions. The result was expressed as the percentage of initial protein
130 concentration.

131 *2.7. Particle size distributions*

132 Particle size distributions of control and oxidised MP were measured with an
133 integrated-laser light scattering instrument (Mastersizer 2000; Malvern Instruments Co.
134 Ltd., Worcestershire, UK). The relative refractive index and absorption were set as
135 1.414 and 0.001, respectively. $D_{4,3}$ is the mean diameter in volume, and $D_{3,2}$ is the mean
136 diameter in surface, called the 'Sauter diameter'. $D_{v,0.5}$ is the size for which 50% of the
137 sample particles have a lower size and 50% have an upper size. The specific surface
138 areas (square metres per gram) were also recorded.

139 *2.8. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis*

140 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was
141 performed on MP according to the method described by Zhou et al. (2014b) with a
142 slight modification. Briefly, the MP suspension (4 mg/mL) was mixed in a 1:1 ratio
143 with 50 mM Tris buffer (8 M urea, 2 M thiourea, 3% (w/v) SDS, 0.05% BPB and 20%
144 (v/v) glycerine, pH 6.8) with or without 75 mM dithiothreitol. The samples were boiled
145 for 4 min before centrifugation (10,000 g, 10 min, 25 °C). Next, 9 μ L of each MP
146 mixture were injected into each gel, comprising a 12% running gel and a 4% stacking
147 gel. The electrophoresis was operated at a constant current of 25 mA using a Mini-
148 PROTEAN 3 Cell apparatus (Bio-Rad Laboratories, Hercules, CA).

149 *2.9. Gel properties*

150 *2.9.1. Preparation of heat-induced gels*

151 For gelation, the control and oxidised MP (30 mg/mL) were prepared for gel property
152 analyses according to the method of Zhou et al. (2014b). The MP suspensions were
153 heated in a water bath from 25 °C to 72 °C at 1 °C/min increments (kept for 5 min at

154 53 °C and 10 min at 72 °C). After heating, the gels were immediately cooled to room
155 temperature.

156 2.9.2. *Gel hardness and WHC*

157 Prior to the hardness measurements, gel samples were allowed to equilibrate at room
158 temperature ($25 \pm 1^\circ\text{C}$) for 1 h. The hardness of MP gels was measured using a cylinder
159 measuring probe (P/0.5S, 12.7 mm) attached to TA-XT Plus Texture Analyzer (Stable
160 Micro Systems Ltd., Godalming, UK) at a constant probe speed of 1.0 mm/s at room
161 temperature. The gel hardness was defined as the initial force required to rupture the
162 gels.

163 WHC values for the gels were determined by a centrifugal method (Xia, Kong, Xiong,
164 & Ren, 2010). Briefly, gel samples (3 g) were centrifuged at 4000 g for 15 min at room
165 temperature. The WHC (%) was expressed using the final weight as a percentage of the
166 weight before centrifugation (6000 g, 15 min, 4 °C).

167 2.9.3. *Microstructure*

168 The surface morphologies of control and oxidised MP gels were examined using field
169 emission scanning electron microscopy (FE-SEM, model S-3400N; Hitachi, Japan) at
170 an accelerator voltage of 15 kV. Cubic samples (approximately $3 \times 3 \times 3$ mm) were
171 prepared and snap-frozen in liquid N₂ (Zhou et al., 2014a). Before imaging, freeze-
172 dried gel samples were mounted on a holder with double-sided adhesive tape and
173 sputter-coated with gold (JFC-1200 fine coater; JEOL, Tokyo, Japan). Sample
174 observation and photomicrography were performed at 500× and 2000× magnifications,
175 respectively.

176 2.9.4. *Low-field NMR relaxation time (T_2)*

177 Low-field NMR relaxation measurements were performed according to a previous
178 method (Zhang, Yang, Tang, Chen, & You, 2015) with some modifications.

179 Approximately 1.6 g of each gel sample formed in a 2 mL screw-cap chromatogram
180 vial were placed inside a cylindrical glass tube (15 mm in diameter) and inserted into
181 the NMR probe of a Niumag Benchtop Pulsed NMR analyser (Niumag PQ001; Niumag
182 Electric Corporation, Shanghai, China). The analyser was operated at 32 °C and a
183 resonance frequency of 18 MHz. The T_2 was measured using the Carr-Purcell-
184 Meiboom-Gill sequence with 4 scans, 8000 echoes, 2.0 s between scans, and 400 μ s
185 between pulses of 90° and 180°. The T_2 relaxation curves were fitted to a multi-
186 exponential curve with the MultiExp Inv Analysis software (Niumag Electric
187 Corporation, Shanghai, China), which used the inverse Laplace transform algorithm.

188 2.10. SPME–GC/MS

189 A stock solution containing all selected flavours (aldehydes and ketones) was freshly
190 prepared in methanol (HPLC grade) and sealed in brown gas-tight glass bottles to
191 prevent volatilising. The stock solution was then pipetted to control and oxidised MP
192 suspensions (30 mg/mL), to a final concentration of 1 mg/kg for each flavour. Each
193 mixture (8 mL protein/control solution + 50 μ L stock solution) was placed in a 20-mL
194 headspace vial and sealed with a polytetrafluoroethylene (PTFE)-faced silicone septum
195 (Supelco, Bellefonte, PA, USA). For gelation, the control and oxidised MP gels were
196 developed according to *Section 2.9.1*. The gel vials were stored at 25 °C for 16 h to
197 allow equilibration.

198 The quantities of flavour compounds present in the headspace of gel vials were
199 determined using solid-phase microextraction (SPME) followed by gas
200 chromatography/mass spectroscopy (GC/MS) analysis according to the procedure
201 described by Zhou et al. (2014a). The SPME parameters were as follows: 75 μ m
202 Carboxen/polydimethylsiloxane (CAR/PDMS) fibre (Supelco, Bellefonte, PA),
203 equilibrated at 45°C for 20 min, extracted at 45°C for 30 min, desorbed at 220°C for 5

204 min. GC-MS conditions: TR-Wax column (30 m × 0.32 mm × 0.25 μm; J&W Scientific,
205 Folsom, CA, USA) was used for separation. The carrier gas was high purity helium at
206 a linear flow rate of 20.4 cm s⁻¹. The initial GC oven temperature was 38°C, held for 6
207 min, rising to 105°C at a rate of 6°C min⁻¹, then raised to 220°C at a rate of 15°C min⁻¹,
208 and held at 220°C for 5 min. The mass spectrometry conditions were electron
209 ionisation (EI) at 70 eV, electron multiplier voltage 350 V, scanning speed 3.00 scans/s,
210 mass range *m/z* 33–350. The results were expressed as the difference in peak areas of
211 flavour compounds between the oxidised MP gels and the control gel, calculated by the
212 following equation (2):

$$213 \quad \text{Free flavour compound (\%)} = (\text{peak area protein/peak area control}) \times 100 \quad (2)$$

214 *2.11. Statistical analysis*

215 Data were expressed as means ± standard deviations of triplicate determinations.
216 Statistical calculation was investigated by analysis of variance using SPSS 17.0 (SPSS,
217 Inc., Chicago, IL). The means were compared using Duncan's multiple range test (*p* <
218 0.05).

219

220 **3. Results and discussion**

221 *3.1. Total and reactive SH groups, surface hydrophobicity, salt solubility and turbidity*

222 The loss of SH groups is one of the primary common characteristics of protein
223 changes under oxidative attack (Xiong et al., 2009). As presented in **Table 1**, compared
224 with the control, the total and reactive SH contents both decreased continuously upon
225 oxidation with the increase of H₂O₂ concentrations (*p* < 0.05). MP are rich in SH groups
226 that can be readily converted to disulphide linkages (S–S) upon oxidative stress (Cao,
227 True, Chen, & Xiong, 2016). The decrease in total and reactive SH contents signified
228 that the SH groups of cysteine were oxidised with the formation of S–S (Sante-

229 Lhoutellier, Aubry, & Gatellier, 2007). Cao et al. (2018) also suggested that SH groups
230 in G-actin were susceptible to hydroxyl radicals and easily changed into intermolecular
231 S–S. Hence, the generated hydroxyl radicals (0.05–5.0 mM H₂O₂) were responsible for
232 the decrease in total and reactive SH contents. In addition, contents of total and reactive
233 SH also significantly decreased ($p < 0.05$) in 0 mM H₂O₂ compared with the control,
234 which may be associated with oxidation induced by Fe³⁺ (Fe³⁺ catalyses H₂O₂ to
235 produce hydroxyl radicals).

236 For monitoring the subtle changes in physical and chemical states of proteins, surface
237 hydrophobicity is a suitable parameter (Sante-Lhoutellier et al., 2007). As shown in
238 **Table 1**, compared with the control, the hydrophobicity of MP (0 mM H₂O₂)
239 significantly increased ($p < 0.05$), implying that the addition of ascorbic acid, Fe³⁺ and
240 oxidative terminators may markedly increase the protein surface hydrophobicity. In
241 addition, surface hydrophobicity of MP gradually increased with the increase of H₂O₂
242 concentrations (0.05–5.0 mM), and the results were similar to those of some previous
243 reports (Cao et al., 2018; Sun et al., 2013). Chelh et al. (2006) suggested that the
244 increase of surface hydrophobicity could be attributed to the unfolding of MP, thus
245 exposing previously buried nonpolar amino acids at their surface. Oxidative damage
246 could induce partial unfolding of MP, thereby exposing the hydrophobic amino acids
247 that were normally buried in protein molecules (Estévez, 2011; Sante-Lhoutellier et al.,
248 2007). Moreover, the cleavage of certain peptides under oxidative stress may also result
249 in the enhancement of surface hydrophobicity (Pacifci, 1987).

250 Salt solubility can reflect the extent of proteins aggregation (Shen et al., 2019). As
251 shown in **Table 1**, compared with the control, salt solubility of MP revealed a slight
252 increase (1.19–3.43%; $p < 0.05$) with low concentrations of oxidant (0–0.2 mM), and
253 then exhibited a rapid decrease (0.59–51.36%; $p < 0.05$) for the further oxidant

254 treatments (0.5–5.0 mM). A slight oxidation could cause a subtle unfolding of proteins
255 (Estévez, 2011), which may lead to better solubility in the salt solution. Nevertheless,
256 with further increase in oxidant, the solubility of MP drastically decreased due to the
257 stronger oxidative attack. This could be explained by the enhancement of surface
258 hydrophobicity and the excessive protein aggregates (Sun, Li, Zhou, Zhao, & Zhao,
259 2014). The exposure of hydrophobic patches or individual groups, aggregation and
260 polymerisation through S–S are all associated with the solubility decrease (Li, Xiong,
261 & Chen, 2012).

262 Turbidity is attributed to the presence of protein aggregates. The turbidity of MP
263 suspensions (**Table 1**) increased ($p < 0.05$) with the increased addition of H₂O₂ (0–5.0
264 mM), which accorded with the loss of SH and the increase of surface hydrophobicity.
265 This indicated that the oxidised incubation could obviously enhance the aggregation
266 behaviour of proteins. Under further analysis, this phenomenon was noted to be caused
267 by the increased surface hydrophobicity due to the exposure of interior hydrophobic
268 amino acid residues and the formation of intra- and intermolecular cross-links under
269 oxidative attack (Li et al., 2012), thus leading to the turbidity increase of MP
270 suspensions.

271 3.2. Particle size distributions

272 Particle size distributions can be used to monitor the aggregation behaviours of
273 proteins under oxidative attack. As shown in **Fig. S1**, compared with the control, the
274 particle size distribution of MP exhibited an obvious shift towards larger particles with
275 the increase of H₂O₂ concentrations. Protein oxidation could promote intermolecular
276 aggregation behaviours between protein molecules, thus increasing the particle size
277 values (Xiong et al., 2009). Specifically, the D_{3,2} value of MP after oxidation showed
278 no significant difference ($p > 0.05$), while the values for other particle sizes (D_{4,3} and

279 $D_{v,0.5}$) (**Table 2**) increased significantly ($p < 0.05$). The large droplets or droplet
280 aggregates have higher weight in the calculation of the $D_{4,3}$ value than they do in the
281 calculation of the $D_{3,2}$ value, and samples with similar $D_{3,2}$, but different $D_{4,3}$ would
282 result primarily from the amount of large droplets or droplet aggregates (Sun et al.
283 2014). This indicates that protein aggregation occurred after oxidation. Moreover, the
284 SH, surface hydrophobicity and turbidity analysis (**Table 1**) conformed to the
285 enhancement of association behaviours between protein molecules with the increase of
286 H_2O_2 concentrations, thereby enlarging the diameter of particles.

287 3.3. SDS-PAGE

288 The aggregation behaviours of the control and oxidised MP were further studied
289 using SDS-PAGE analysis (**Fig. S2**). In the absence of dithiothreitol, a large polymer
290 appeared at the top of the stack gel (**Fig. S2A**), suggesting the aggregation of MP. In
291 addition, MP aggregates induced by oxidative attack could form much larger aggregates
292 that could not enter the gel. Moreover, the intensities of the aggregation bands and the
293 aggregates all decreased and were not recovered in the presence of dithiothreitol (**Fig.**
294 **S2B**). This phenomenon indicated that the cross-links of the MP induced by oxidation
295 were not only through S–S but also through other covalent bonds (Cao et al., 2016). As
296 shown in the comparison between **Fig. S2A** and **Fig. S2B**, myosin heavy chain (MHC),
297 α -actinin and actin participated in the formation of aggregates.

298 3.4. Gel properties

299 3.4.1. Hardness and WHC

300 As aforementioned, oxidation had a pronounced effect on the physicochemical states
301 of MP. The hardness (**Fig. 1A**) and WHC (**Fig. 1B**) of the control and oxidised MP gels
302 were further evaluated. The mean hardness of the control gel was 21.9 ± 1.4 g and this
303 value was similar to that found by Xu, Han, Fei, and Zhou (2011). Compared with the

304 control, the hardness of mildly oxidised MP gels (0–0.2 mM H₂O₂) increased
305 significantly ($p < 0.05$). Due to the slightly unfolding of MP (salt solubility analysis), a
306 firmer matrix structure of MP gels may be formed during the heating process. Xiong et
307 al. (2010) also suggested that mild oxidation could promote protein network formation
308 and enhance the gelation of MP. Nevertheless, with more addition of H₂O₂ (0.5–5.0
309 mM), a significant decrease ($p < 0.05$) in gel hardness was observed, implying a weak
310 gel structure. In particular, heat-induced S–S bonds were regarded as an important
311 supporting force for the matrix structure of protein gels (Xiong et al., 2010). Hence, the
312 decrease of hardness may be attributed to the reduction of SH contents in MP (**Table**
313 **1**), thus leading to disordered aggregation replacing the ordered cross-links during
314 heating. Moreover, the decrease of salt solubility of MP could limit the ordered cross-
315 links within protein molecules during gelation.

316 Furthermore, WHC gave a quantitative indication of the amount of water maintained
317 within the protein gel structure, and this could reflect aspects of the spatial structure of
318 the gel (Zhou et al., 2014b). As shown in **Fig. 1B**, the control gel had the strongest
319 ability to retain water, and the WHC abilities of oxidised MP gels were gradually
320 weakened ($p < 0.05$) with an increase of H₂O₂ concentration (0–5.0 mM). As reported,
321 the structural integrity of myosin is of paramount importance for gelation and water
322 holding in meat (Deng et al., 2010). Oxidative damage to myosin could result in an
323 inferior gel network formation, causing lower elasticity with poor WHC in the gel
324 matrix. This was in good agreement with the surface hydrophobicity of MP (**Table 1**),
325 which could also reflect the affinity of proteins with water.

326 *3.4.2. Microstructure*

327 The changes in MP gel properties induced by oxidation were further investigated by
328 the measurements of surface morphologies, and the results are presented in **Fig. 2**. The

329 control gel appeared as a flat surface with several visible pores (**Fig. 2A** and **2B**), which
330 indicated an ordered network structure due to protein stretching. At 2000 times
331 magnification, the protein (control gel) exhibits a good cross-linked structure.
332 Compared to the control, the mildly oxidised MP gels (0–0.2 mM H₂O₂) exhibited some
333 fragments on the gel surface and a lower number of visible pores (**Fig. 2B**). The pore
334 size of these gel was significantly reduced ($\times 500$), and the original cross-linked
335 structure was transformed into an aggregated particle structure, and the roughness is
336 increased ($\times 2000$), thereby yielding a firm pattern (hardness analysis). This
337 phenomenon was attributed to the slight shrinkage of MP upon oxidation (Astruc,
338 Gatellier, Labas, Lhoutellier, & Marinova, 2010). As a continuous increase of H₂O₂
339 (0.5–1.0 mM), aggregated particles and roughness further increased with the much
340 more smaller pore size. Under 5 mM H₂O₂, the pores disappeared, and the fragments
341 aggregated into clump structures. The surface morphologies of these gels revealed an
342 uneven dense structure with aggregates and increased the roughness of the overlapped
343 surface. This trend was in accordance with a previous observation (Zhou et al., 2014a).
344 Formation of the surface structures of oxidised MP gels described previously could be
345 attributed to the intra- and intermolecular cross-links and aggregates within proteins
346 induced by oxidative modification (Sun et al., 2014), thus leading to a disordered gel
347 network structure during the heating process. In addition, the clump structures clearly
348 aggregated into larger globular clusters when subjected to higher oxidative attack (2.5–
349 5.0 mM H₂O₂), indicating an accelerated state of aggregation (**Fig. 2B**). These structural
350 features implied that proteins under higher oxidative stress could undergo more steric
351 modifications and contribute to the layered surface with bunched aggregation (Zhou et
352 al., 2014a). These micrographs demonstrated the significant influences of oxidative
353 treatments on protein gels. Moreover, these observations were in agreement with the

354 results of SH contents, surface hydrophobicity, turbidity and particle size distributions
355 (**Table 1** and **Fig. S1**).

356 *3.4.3. Relaxation time analysis*

357 A fitted T_2 distribution was used to assess the relaxation time of hydrogen protons.
358 The T_2 relaxation time distributions for the control and oxidised MP gels are shown in
359 **Fig. 3**. Three peaks were noted for the control and in the gels with lower H_2O_2
360 concentrations (0–1.0 mM), while four peaks were noted for the gels with 2.5–5.0 mM
361 H_2O_2 . This meant that the protein gels under different oxidation levels could restrict
362 water mobility at different magnitudes (Wang, Zhang, Bhandari, & Gao, 2016). The
363 components with shorter relaxation time, T_{2b} and T_{21} (0–10 ms), represented the protons
364 in macromolecular structures and those combined closely with the macromolecular
365 structures, respectively. The component T_{22} (10–100 ms) was assigned to myofibrillar
366 water and water within the protein structure. The last peak represented the extra-
367 myofibrillar water (T_{23}) population, and this peak appeared between 300 and 2000 ms.
368 Generally, the existence of four groups of water in MP gels was in agreement with some
369 previous reports (Wang et al., 2016; Zheng et al., 2015).

370 With oxidation treatments, the relaxation time of T_{22} increased from 14.17 ms
371 (control gel) to 16.30–28.48 ms (oxidised gels) with increasing H_2O_2 concentrations,
372 and T_{23} increased from 613.59 ms (control and oxidised gels with 0–1.0 mM H_2O_2) to
373 705.48 ms (oxidised gels with 2.5–5.0 mM H_2O_2) (**Table 1S**). If the relaxation time is
374 shorter, a smaller amount of mobile water is available, whereas the longer relaxation
375 time implies a more mobile water fraction (Shao et al., 2016). Hence, the increased T_2
376 relaxation times suggested that oxidative modifications could lead to a certain level of
377 the immobilised water shifting to free water. This may be associated with the
378 enhancement in hydrophobic trend arising from the increased surface hydrophobicity

379 of MP (**Table 1**). Also, the increased aggregation behaviours (**Fig. S1** and **Table 2**)
380 within MP molecules could decrease the surface areas in gels, thus resulting in a
381 decrease of macromolecule sites for WHC (Wang et al., 2016). Moreover, the relaxation
382 component T_{2b} had divided into another part (T_{21}) at higher H_2O_2 concentrations (2.5–
383 5.0 mM). This may be due to the degradation of MP upon higher oxidative stress, thus
384 contributing to greater mobility of the water in macromolecular structures. In addition,
385 McDonnell et al. (2013) suggested that certain side-chains, such as carboxyl-, amino-,
386 hydroxyl-, sulfhydryl-groups, and even carbonyl- and imido-groups, in proteins were
387 responsible for water binding. Therefore, the changes in T_{2b} relaxation time
388 corresponded to the availability of protein side-chains, as related to the oxidative
389 modifications. These phenomena accorded with the WHC results (**Fig. 1B**).

390 *3.5. Binding of MP gels to flavours*

391 A homologous series of aldehydes and ketones that varied in chain length was
392 selected to investigate the binding performances of MP gels under different oxidation
393 levels. In protein solution systems, the longer the carbon chain, the stronger the ability
394 of the flavour compound to bind to the protein. (Lou, Yang, Sun, Pan, & Cao, 2017;
395 Zhou et al., 2014a). By contrast, in this study, the free percentages of aldehydes and
396 ketones in each vial increased with the increase in carbon chain length (**Fig. 4**),
397 suggesting it was more difficult for the flavour compounds with longer chain length to
398 bind with MP gels, which can be related to the steric effect of the gel network structure.
399 In addition, consistent with the finding reported by Wang and Arntfield (2015), the free
400 percentage of all aldehydes was lower than that of the ketones with the same carbon
401 numbers in all vials (**Fig. 4**), suggesting that the binding abilities of oxidised MP gels
402 to aldehydes were stronger than to ketones. This was because of the higher molecular

403 activities and lower steric hindrance effects of carbonyl groups in aldehydes (Kühn et
404 al., 2008).

405 As shown in Fig. 4A, the free percentages of hexanal and heptanal first decreased
406 and then increased significantly ($p < 0.05$) with increased oxidation levels (0–5.0 mM).
407 Although the free percentages of pentanal and octanal showed no significant changes
408 ($p > 0.05$) at different oxidation levels, their trends were similar to those of hexanal and
409 heptanal. The free percentages of ketones (Fig. 4B) (except for 2-octanone) showed no
410 significant changes at different oxidation levels (0–5.0 mM). Volatile release from food
411 is primarily controlled by the following two factors: the nature of aroma compounds
412 (such as volatility and polarity) and the resistance to mass transfer from a food matrix
413 to the air phase (Mao, Roots, & Miao, 2015). Protein oxidation increased the surface
414 hydrophobicity and interaction of proteins (**Table 1**); the oxidised MP gels could
415 exhibit higher binding phenomena through stronger interaction force (such as
416 hydrophobic interaction) to flavours, thus reducing their free percentages in the
417 headspace. However, the binding of a certain protein gel to flavours may also be
418 influenced by the steric hindrance effect or mass transfer from MP gel. Therefore,
419 flavours with a longer carbon chain could produce a stronger steric hindrance effect due
420 to the gel network, thus preventing their access to the interior hydrophobic binding sites
421 and thus increasing their presence in the headspace (Wang & Arntfield, 2015). As
422 shown in **Fig. 2**, as the degree of oxidation increased, the homogeneous cross-linked
423 MP gel network gradually changes to a granular shape, and gradually loses the gel
424 network structure (the pore size of the gel network also gradually decreases), which
425 increases the steric resistance of the MP gel, increasing the content of aroma
426 components in the headspace vial. Therefore, the release of flavour compounds

427 depended on the balance between protein–flavour compound interactions and gel
428 network–flavour compound limitations (Mao et al., 2014).

429 **4. Conclusions**

430 The influence of oxidative modifications induced by H₂O₂ (0–5.0 mM) on the
431 properties of MP and MP gels was investigated. With an increase in H₂O₂
432 concentrations, MP tended to expose their interior hydrophobic amino acids and to lose
433 SH content, thus leading to enhanced aggregation behaviours, an increase in surface
434 hydrophobicity and turbidity and a larger particle size distribution. The covalent bonds
435 in the MP aggregates included S–S, among others. Under oxidative attack, MP gels
436 demonstrated decreased WHC and more mobility of water. Mild oxidation (0–0.5 mM
437 H₂O₂) slightly increased the salt solubility of MP and the hardness of MP gels. The
438 abilities of MP gels to bind to aldehydes and ketones decreased with the growth of the
439 carbon chain. The release of flavours in a gel was different from that in a protein
440 solution due to mass transfer from a gel matrix to the air phase and heat treatment.
441 Under the balance between protein–flavour compound interaction and gel network–
442 flavour compound steric hindrance, MP oxidation only significantly affected ($p < 0.05$)
443 the binding between MP gels and hexanal, heptanal, and 2-octanone, while other
444 flavour compounds were not affected significantly ($p > 0.05$).

445

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452

453 **References**

- 454 Astruc, T., Gatellier, P., Labas, R., Lhoutellier, V. S., & Marinova, P. (2010).
455 Microstructural changes in *m. rectus abdominis* bovine muscle after heating. *Meat*
456 *Science*, 85(4), 743–751.
- 457 Boland, A. B., Delahunty, C. M., & van Ruth, S. M. (2006). Influence of the texture of
458 gelatin gels and pectin gels on strawberry flavour release and perception. *Food*
459 *Chemistry*, 96(3), 452–460.
- 460 Cao, Y., True, A. D., Chen, J., & Xiong, Y. L. (2016). Dual Role (Anti- and Pro-oxidant)
461 of Gallic Acid in Mediating Myofibrillar Protein Gelation and Gel *in Vitro*
462 Digestion. *Journal of Agricultural and Food Chemistry*, 64(15), 3054–3061.
- 463 Chelh, I., Gatellier, P., & Santé-Lhoutellier, V. (2006). Technical note: A simplified
464 procedure for myofibril hydrophobicity determination. *Meat Science*, 74(4), 681–
465 683.
- 466 Deng, Y., Rosenvold, K., Karlsson, A. H., Horn, P., Hedegaard, J., Steffensen, C. L.,
467 & Andersen, H. J. (2010). Relationship Between Thermal Denaturation of Porcine
468 Muscle Proteins and Water-holding Capacity. *Journal of Food Science*, 67(5),
469 1642–1647.
- 470 Estévez, M. (2011). Protein carbonyls in meat systems: A review. *Meat Science*, 89(3),
471 259–279.
- 472 Gierczynski, I., Guichard, E., & Laboure, H. (2011). Aroma perception in dairy
473 products: the roles of texture, aroma release and consumer physiology. A
474 review. *Flavour and Fragrance Journal*, 26(3), 141–152.
- 475 Guichard, E. (2002). Interactions between flavor compounds and food ingredients and
476 their influence on flavor perception. *Food Reviews International*, 18(1), 49–70.

477 Guinard, J. X., & Marty, C. (1995). Time-intensity measurement of flavor release from
478 a model gel system: effect of gelling agent type and concentration. *Journal of Food*
479 *Science*, 60(4), 727–730.

480 Hansson, A., Giannouli, P., & van Ruth, S. (2003). The influence of gel strength on
481 aroma release from pectin gels in a model mouth and *in vivo*, monitored with
482 proton-transfer-reaction mass spectrometry. *Journal of Agricultural and Food*
483 *Chemistry*, 51(16), 4732–4740.

484 Juteau-Vigier, A., Atlan, S., Deleris, I., Guichard, E., Souchon, I., & Trelea, I. C. (2007).
485 Ethyl hexanoate transfer modeling in carrageenan matrices for determination of
486 diffusion and partition properties. *Journal of Agricultural and Food*
487 *Chemistry*, 55(9), 3577–3584.

488 Kühn, J., Considine, T., & Singh, H. (2008). Binding of flavor compounds and whey
489 protein isolate as affected by heat and high pressure treatments. *Journal of*
490 *Agricultural and Food Chemistry*, 56(21), 10218–10224.

491 Lee, H. A., Choi, S. J., & Moon, T. W. (2006). Characteristics of sodium caseinate- and
492 soy protein isolate-stabilized emulsion-gels formed by microbial transglutaminase.
493 *Journal of Food Science*, 71(6), C352–C357.

494 Li, C., Xiong, Y. L., & Chen, J. (2012). Oxidation-induced unfolding facilitates Myosin
495 cross-linking in myofibrillar protein by microbial transglutaminase. *Journal of*
496 *Agricultural & Food Chemistry*, 60(32), 8020–8027.

497 Lou, X., Yang, Q., Sun, Y., Pan, D., & Cao, J. (2017). The effect of microwave on the
498 interaction of flavour compounds with G-actin from grass carp
499 (*Ctenopharyngodon idella*). *Journal of the Science of Food and Agriculture*,
500 97(12), 3917–3922.

501 Mao, L., Roos, Y. H., & Miao, S. (2014). Study on the rheological properties and
502 volatile release of cold-set emulsion-filled protein gels. *Journal of Agricultural*
503 *and Food Chemistry*, 62(47), 11420–11428.

504 Mao, L., Roos, Y. H., & Miao, S. (2015). Effect of maltodextrins on the stability and
505 release of volatile compounds of oil-in-water emulsions subjected to freeze–thaw
506 treatment. *Food Hydrocolloids*, 50, 219–227.

507 McDonnell, C. K., Allen, P., Duggan, E., Arimi, J. M., Casey, E., Duane, G., & Lyng,
508 J. G. (2013). The effect of salt and fibre direction on water dynamics, distribution
509 and mobility in pork muscle: a low field NMR study. *Meat Science*, 95(1), 51–58.

510 O'Neill, T. E., & Kinsella, J. E. (1987). Binding of alkanone flavors to β -lactoglobulin:
511 effects of conformational and chemical modification. *Journal of Agricultural and*
512 *Food Chemistry*, 35(5), 770–774.

513 Pacifici, R. E. (1987). Protein damage and degradation by oxygen radicals. *Journal of*
514 *Biological Chemistry*, 262(20), 9902–9907.

515 Sante-Lhoutellier, V., Aubry, L., & Gatellier, P. (2007). Effect of oxidation on *in vitro*
516 digestibility of skeletal muscle myofibrillar proteins. *Journal of Agricultural &*
517 *Food Chemistry*, 55(13), 5343–5348.

518 Shao, J. H., Deng, Y. M., Song, L., Batur, A., Jia, N., & Liu, D. Y. (2016). Investigation
519 the effects of protein hydration states on the mobility water and fat in meat batters
520 by LF-NMR technique. *LWT - Food Science and Technology*, 66, 1–6.

521 Shen, H., Zhao, M., & Sun, W. (2019). Effect of pH on the interaction of porcine
522 myofibrillar proteins with pyrazine compounds. *Food Chemistry*, 287, 93–99.

523 Sun, W., Li, Q., Zhou, F., Zhao, H., & Zhao, M. (2014). Surface characterization of
524 oxidized myofibrils using X-ray photoelectron spectroscopy and scanning electron
525 microscopy. *Journal of Agricultural & Food Chemistry*, 62(30), 7507–7514.

526 Sun, W., Zhou, F., Sun, D.-W., & Zhao, M. (2013). Effect of Oxidation on the
527 Emulsifying Properties of Myofibrillar Proteins. *Food and Bioprocess Technology*,
528 6(7), 1703–1712.

529 Wang, K., & Arntfield, S. D. (2015). Binding of selected volatile flavour mixture to
530 salt-extracted canola and pea proteins and effect of heat treatment on flavour
531 binding. *Food Hydrocolloids*, 43, 410–417.

532 Wang, K., & Arntfield, S. D. (2016). Modification of interactions between selected
533 volatile flavour compounds and salt-extracted pea protein isolates using chemical
534 and enzymatic approaches. *Food Hydrocolloids*, 61, 567–577.

535 Wang, L., Zhang, M., Bhandari, B., & Gao, Z. (2016). Effects of malondialdehyde-
536 induced protein modification on water functionality and physicochemical state of
537 fish myofibrillar protein gel. *Food Research International*, 86, 131–139.

538 Xia, X., Kong, B., Xiong, Y., & Ren, Y. (2010). Decreased gelling and emulsifying
539 properties of myofibrillar protein from repeatedly frozen-thawed porcine
540 longissimus muscle are due to protein denaturation and susceptibility to
541 aggregation. *Meat Science*, 85(3), 481–486.

542 Xiong, Y., Blanchard, S. P., Ooizumi, T., & Ma, Y. (2010). Hydroxyl radical and ferryl-
543 generating systems promote gel network formation of myofibrillar protein.
544 *Journal of Food Science*, 75(2), C215–C221.

545 Xiong, Y. L., Park, D. K., & Ooizumi, T. (2009). Variation in the cross-linking pattern
546 of porcine myofibrillar protein exposed to three oxidative environments. *Journal*
547 *of Agricultural & Food Chemistry*, 57(1), 153–159.

548 Xu, X. L., Han, M. Y., Fei, Y., & Zhou, G. H. (2011). Raman spectroscopic study of
549 heat-induced gelation of pork myofibrillar proteins and its relationship with
550 textural characteristic. *Meat Science*, 87(3), 159–164.

- 551 Zhang, Z., Yang, Y., Tang, X., Chen, Y., & You, Y. (2015). Chemical forces and water
552 holding capacity study of heat-induced myofibrillar protein gel as affected by high
553 pressure. *Food Chemistry*, *188*, 111–118.
- 554 Zheng, H., Xiong, G., Han, M., Deng, S., Xu, X., & Zhou, G. (2015). High
555 pressure/thermal combinations on texture and water holding capacity of chicken
556 batters. *Innovative Food Science & Emerging Technologies*, *30*, 8–14.
- 557 Zhou, F., Zhao, M., Su, G., & Sun, W. (2014b). Binding of aroma compounds with
558 myofibrillar proteins modified by a hydroxyl-radical-induced oxidative system.
559 *Journal of Agricultural & Food Chemistry*, *62*(39), 9544–9552.
- 560 Zhou, F., Zhao, M., Zhao, H., Sun, W., & Cui, C. (2014a). Effects of oxidative
561 modification on gel properties of isolated porcine myofibrillar protein by peroxy
562 radicals. *Meat Science*, *96*(4), 1432–1439.

563 **Table 1** Total and reactive sulfhydryl groups, surface hydrophobicity, salt solubility and turbidity of the control and oxidised MP

| H ₂ O ₂ (mM) | Total sulfhydryl (nmol/mg protein) | Reactive sulfhydryl (nmol/mg protein) | Surface hydrophobicity (µg BPB) | Salt solubility (%) | Turbidity (FTU) |
|------------------------------------|---------------------------------------|--|------------------------------------|------------------------|--------------------|
| Control | 9.02 ± 0.12g | 8.45 ± 0.09h | 22.75 ± 1.22a | 83.57 ± 1.39d | 83.11 ± 0.82a |
| 0.0 | 8.30 ± 0.04f | 6.35 ± 0.05g | 38.02 ± 0.86bc | 84.57 ± 0.46de | 84.27 ± 0.58a |
| 0.05 | 8.19 ± 0.02f | 5.99 ± 0.07f | 36.57 ± 2.15b | 86.43 ± 1.09e | 87.13 ± 0.56b |
| 0.2 | 7.99 ± 0.05e | 5.56 ± 0.11e | 38.59 ± 0.85c | 86.39 ± 0.66e | 90.44 ± 2.37c |
| 0.5 | 6.95 ± 0.03d | 3.24 ± 0.11d | 41.26 ± 0.72d | 83.08 ± 0.94d | 91.71 ± 1.99c |
| 1.0 | 5.87 ± 0.03c | 2.84 ± 0.07c | 42.47 ± 0.34d | 79.28 ± 2.73c | 92.74 ± 0.91c |
| 2.5 | 3.52 ± 0.11b | 1.82 ± 0.05b | 46.23 ± 0.71e | 52.23 ± 0.96b | 97.05 ± 1.47d |
| 5.0 | 1.01 ± 0.05a | 0.37 ± 0.03a | 49.88 ± 1.08f | 40.65 ± 1.02a | 96.70 ± 0.90d |

564 Values in the same column with different letters were significantly different ($p < 0.05$).

565 **Table 2** Average particle size ($D_{3,2}$ and $D_{4,3}$) and span of the control and oxidised MP

| H ₂ O ₂ (mM) | $D_{3,2}$ (μm) | $D_{4,3}$ (μm) | $D_{v,0.5}$ (μm) | Span |
|------------------------------------|-----------------------------|-----------------------------|-------------------------------|-------------------|
| Control | 41.55 \pm 1.51a | 99.58 \pm 2.76a | 69.57 \pm 1.63a | 2.89 \pm 0.05ab |
| 0.0 | 50.93 \pm 0.84b | 119.53 \pm 1.09b | 86.79 \pm 1.30b | 2.80 \pm 0.09a |
| 0.05 | 51.47 \pm 1.80b | 120.12 \pm 3.39bc | 86.18 \pm 3.36b | 2.84 \pm 0.06a |
| 0.2 | 51.06 \pm 1.58b | 121.22 \pm 3.70bc | 87.00 \pm 3.40b | 2.86 \pm 0.03a |
| 0.5 | 51.53 \pm 1.19b | 125.19 \pm 2.46bc | 87.57 \pm 2.03b | 3.01 \pm 0.03bc |
| 1.0 | 48.80 \pm 1.90b | 126.49 \pm 4.66c | 87.62 \pm 3.70b | 3.11 \pm 0.03cd |
| 2.5 | 50.72 \pm 2.12b | 138.31 \pm 4.05d | 96.93 \pm 3.48c | 3.12 \pm 0.06cd |
| 5.0 | 51.77 \pm 2.21b | 146.12 \pm 5.07e | 101.82 \pm 4.95c | 3.18 \pm 0.17d |

566 Values in the same column with different letters were significantly different ($p < 0.05$).

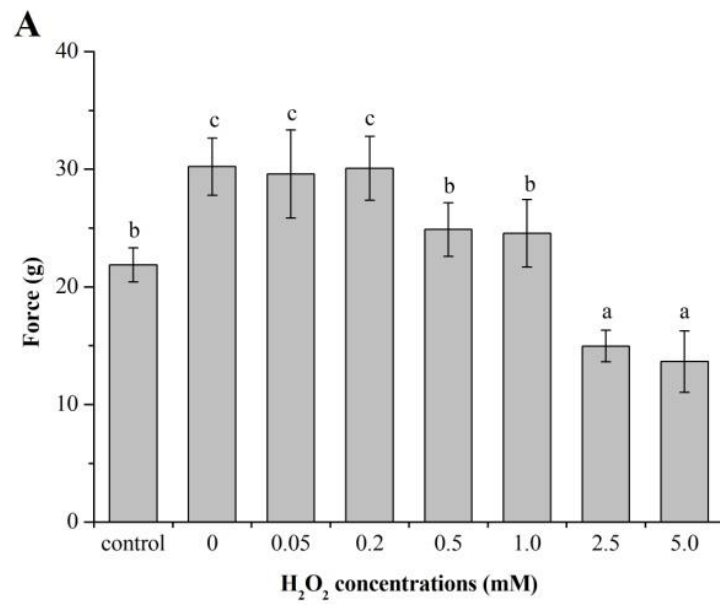
567 **Figure Captions:**

568 **Fig. 1.** Hardness (A) and WHC (B) of the control and oxidised MP gels. Different letters
569 denote a significant difference between means ($p < 0.05$).

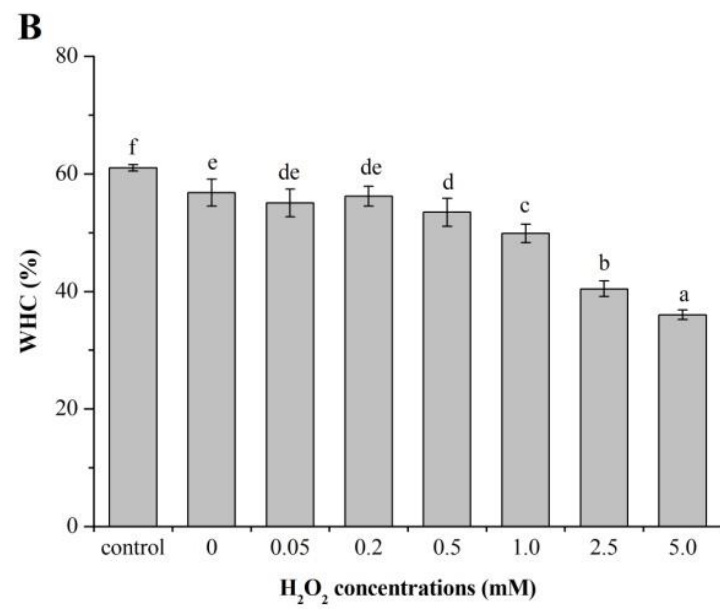
570 **Fig. 2.** Scanning electron microscope micrographs at $\times 500$ (A) and $\times 2000$ (B)
571 magnification of the control and oxidised MP gels. Scale bars indicate $100\ \mu\text{m}$ (A) and
572 $20\ \mu\text{m}$ (B).

573 **Fig. 3.** Distributions of T_2 relaxation times of the control and oxidised MP gels.

574 **Fig. 4.** Binding phenomena of the control and oxidised MP gels to selected aldehydes
575 (A) and ketones (B). Results were expressed as percentage of free flavours found in the
576 headspace of the control gel. Capital letters denote significant differences ($p < 0.05$) in
577 flavour compound release for a same oxidant concentration, while lowercase letters
578 denote significant differences ($p < 0.05$) in compound release between oxidant
579 concentrations.

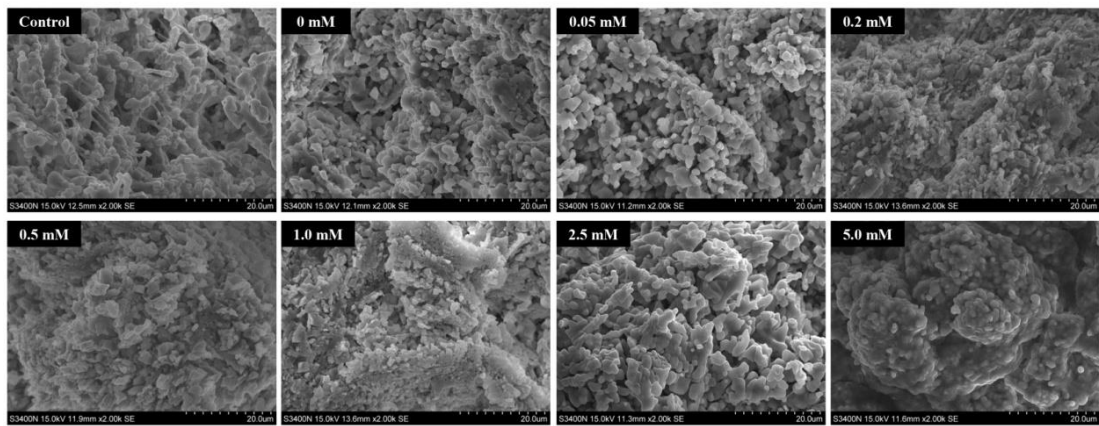
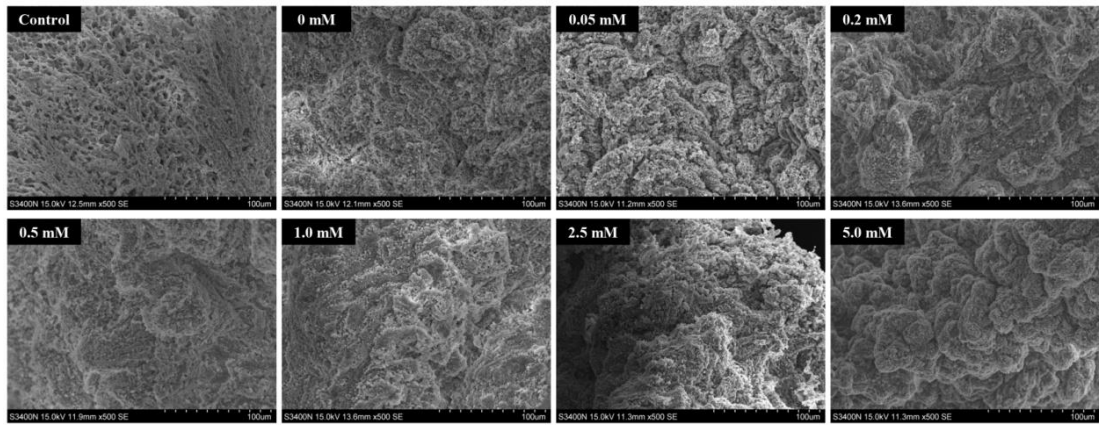


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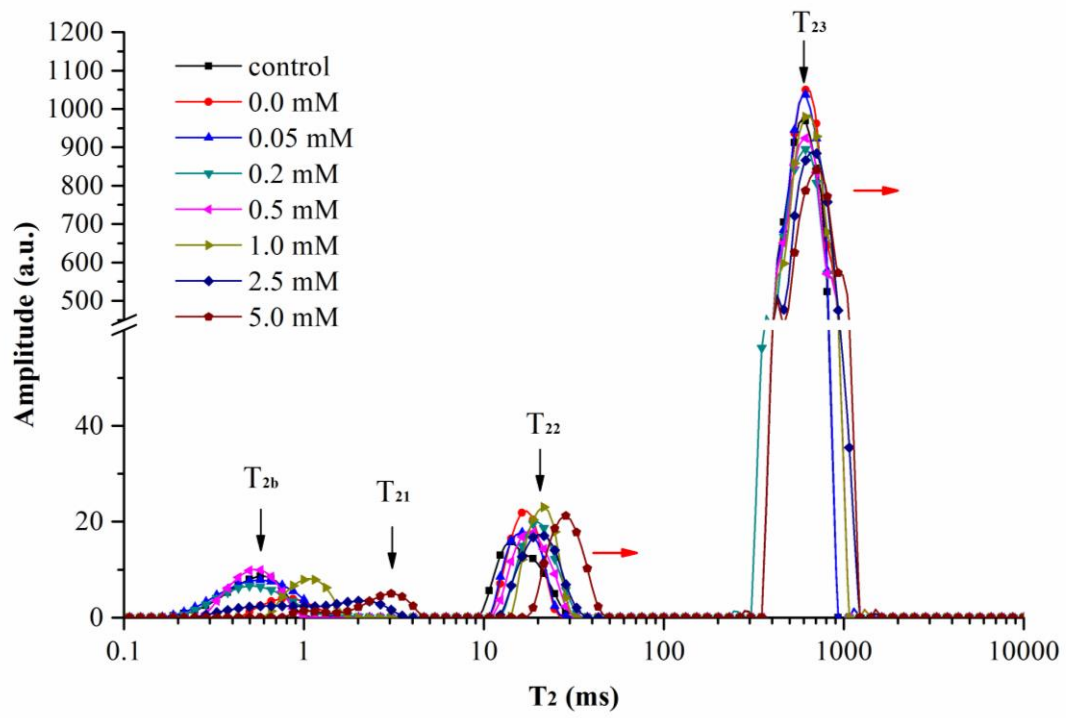
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582 **Fig. 1**



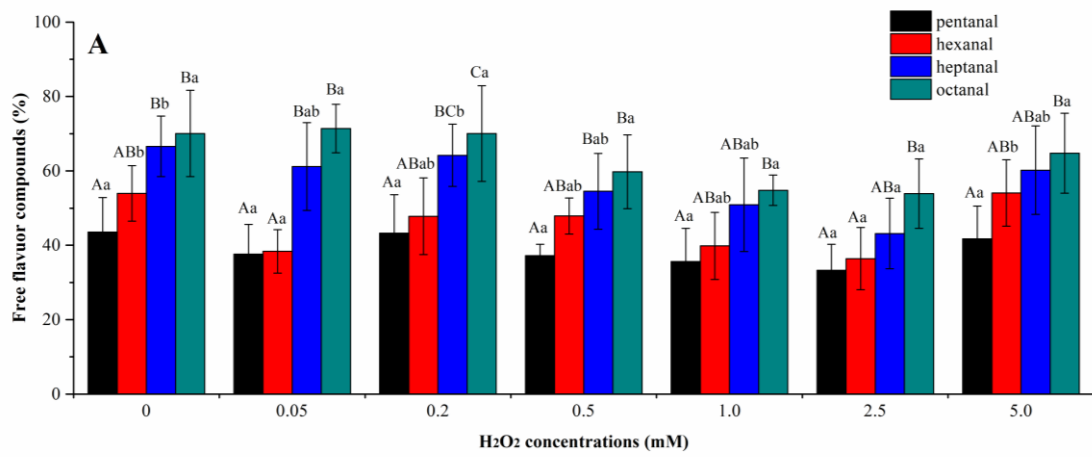
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584 **Fig. 2**

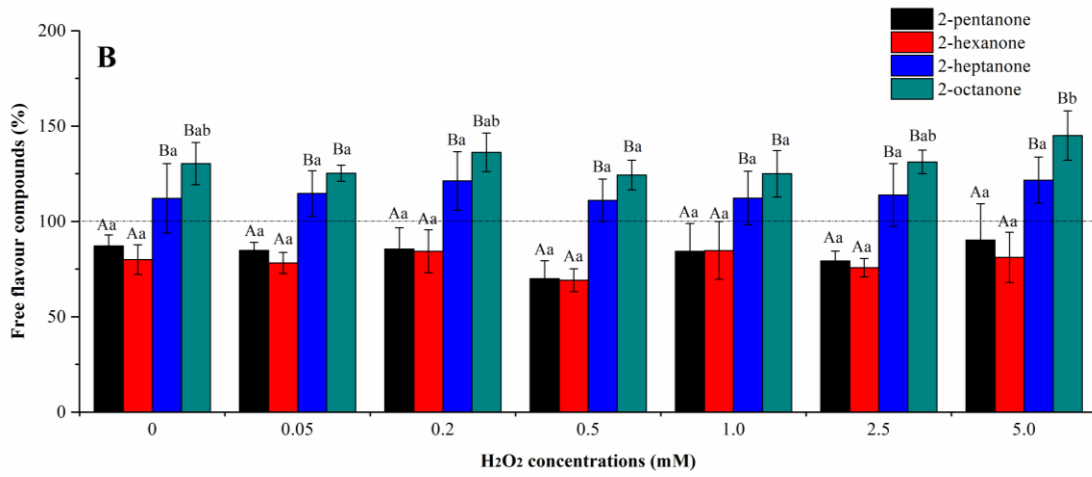


585

586 **Fig. 3**



587



588

589 **Fig. 4**