

## Simultaneous estimation of scavenging capacities of peach extract for multiple reactive oxygen species by fluorescence fingerprint method

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Abstract: In this study, the potential of using fluorescence fingerprint, also known as fluorescence excitation-emission matrix, for estimating the scavenging capacity of peach extract on reactive oxygen species (ROS) was investigated. Samples from each of the five cultivars (Asama Hakuto, Hakuho, Kawanakajima Hakuto, Natsukko and Ougonto) were freeze-dried and crushed. The scavenging capacities of peach extracts for the target ROS (hydroxyl, superoxide, alkoxyl radicals and singlet oxygen) were measured by electron paramagnetic resonance spin trapping method. Fluorescence fingerprints of the same samples were obtained. Partial least squares regression analysis was carried out to develop prediction models for ROS scavenging capacity. The models were assessed by external validation. Fluorescence fingerprint was found to accurately estimate the scavenging capacity for the alkoxyl and superoxide radicals with the prediction error of 0.06 mmol trolox eq./mL and 0.31 mmol  $\alpha$ -lipoic acid eq./mL with a coefficient of determination of prediction (R<sup>2</sup>P) of 0.78 and 0.91, respectively.

March 21, 2017

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Dear *Food Chemistry* Editorial Board,

I enclose our manuscript entitled *Simultaneous estimation of scavenging capacities of peach extract for multiple reactive oxygen species using fluorescence fingerprint method*, which is authored by Vipavee Trivittayasil, Hiromi Kameya, Toshihiko Shoji, Mizuki Tsuta, Mito Kokawa and Junichi Sugiyama, for your kind consideration of its suitability for publication in *Food Chemistry*. It contains original research and has not been considered for publication in any journal.

This paper investigated the capacity of using fluorescence fingerprint (FF) to simultaneously estimate scavenging capacities of peach extract for multiple reactive oxygen species. The scavenging capacity for each target reactive oxygen species (hydroxyl, superoxide, alkoxy radicals and singlet oxygen) was measured by electron paramagnetic resonance (EPR) spin trapping method. FF has shown a potential use for estimating well scavenging capacity for alkoxy and superoxide radicals. The components corresponding to the good estimation were also identified. This finding suggests FF as a potential method for assessing the scavenging capacity of food products for quality assurance application.

Sincerely yours,

Mizuki TSUTA

Editor: still there are some modifications to be performed in the paper. Authos SHOULD PAY ATTENTION to all of them and resubmit:

Line 88: procyanidins are polyphenols. Polyphenols do not represent a class of phenolic compounds. Authors should write: 'The scavenging capacities of phenolic compounds commonly found in peach were also measured to'....

The sentence has been changed as suggested. (Line 89)

Line 164: 0.4 mol/L

The unit has been changed as suggested. (Line 163)

Line 173: acetone should be 'propanone'

acetone has been changed to propanone as suggested. (Line 172)

Line 190: ul should be uL

This has been changed as suggested. (Line 189)

Table 1: Statistical analyses to compare the cultivars should be carried out

ANOVA with post-hoc Tukey's honest significance test was used to compare the variables between cultivars. The result was added to Table 1 and the the method added to Statistical Analysis section of methodology. (Line 249)

Units: 100 g and not 100g

This has been changed as suggested. (Table 1)

### Highlights

- The scavenging capacities of peach extract for RO,  $O_2^-$  and  $^1O_2$  but not OH by peach are correlated.
- FF could estimate the scavenging capacity of peach extract for RO and  $O_2^-$ .
- Fluorescence peak of procyanidins is considered to be important for the prediction.

1 **Simultaneous estimation of scavenging capacities of peach extract for multiple**  
2 **reactive oxygen species by fluorescence fingerprint method**

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22

23 **Abstract**

24 In this study, the potential of using fluorescence fingerprint, also known as fluorescence  
25 excitation–emission matrix, for estimating the scavenging capacity of peach extract on  
26 reactive oxygen species (ROS) was investigated. Samples from each of the five  
27 cultivars (Asama Hakuto, Hakuho, Kawanakajima Hakuto, Natsukko and Ougonto)  
28 were freeze-dried and crushed. The scavenging capacities of peach extracts for the  
29 target ROS (hydroxyl, superoxide, alkoxy radicals and singlet oxygen) were measured  
30 by electron paramagnetic resonance spin trapping method. Fluorescence fingerprints of  
31 the same samples were obtained. Partial least squares regression analysis was carried  
32 out to develop prediction models for ROS scavenging capacity. The models were  
33 assessed by external validation. Fluorescence fingerprint was found to accurately  
34 estimate the scavenging capacity for the alkoxy and superoxide radicals with the  
35 prediction error of 0.06 mmol trolox eq./mL and 0.31 mmol  $\alpha$ -lipoic acid eq./mL with a  
36 coefficient of determination of prediction ( $R^2_P$ ) of 0.78 and 0.91, respectively.

37

38 Keywords: Fluorescence Fingerprint; electron paramagnetic resonance (EPR);  
39 scavenging capacity; peach; procyanidins

40

41

## 42 **1.Introduction**

43 Reactive oxygen species (ROS) are implicated in aging and many life-threatening  
44 diseases such as cancers, cardiovascular diseases and neurological disorders (Brieger,  
45 Schiavone, Miller, & Krause, 2012). To counterbalance the effects of ROS during  
46 abnormal oxidative stress, dietary antioxidant supplements are recommended as they  
47 could prevent some ROS–induced disorders (Poljsak, Suput, & Milisav, 2013). Although  
48 the benefits of consuming antioxidant supplements are still widely debated (Blomhoff,  
49 2005; Stanner, Hughes, Kelly, & Buttriss, 2004), foods rich in antioxidant capacity  
50 nevertheless hold considerable interest (Shahidi & Ambigaipalan, 2015).

51

52 An established method of directly measuring ROS is electron paramagnetic resonance  
53 (EPR) spin trapping. Highly unstable ROS are trapped with a specific compound called  
54 a spin trap to form adducts, which are stable for a sufficiently long time to be detected  
55 using EPR (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002). With  
56 increasing interest in functional foods, there are movements such as the new legislation  
57 for functional foods in Japan that encourages labeling of functional properties on food  
58 products (Amagase, 2015). Such a trend has led to the need for a simpler method of  
59 assessing the scavenging activity of food products for quality assurance applications as  
60 EPR is not only costly but also requires skilled technicians and test reagents for its  
61 operation. Fluorescence fingerprint (FF), also known as fluorescence excitation–  
62 emission matrix (EEM), is a nontargeted technique that has been gathering attention in  
63 recent years due to its high selectivity for a spectroscopic method and simple sample  
64 preparation. FF is a powerful tool as it provides all spectral data, consisting of  
65 fluorescence intensities at wide excitation and fluorescence emission wavelengths.

66 Many of the chemicals that are reported to be related to antioxidant capacity (oxygen  
67 radical absorbance capacity; ORAC) such as total phenolic content and total  
68 anthocyanin (Prior et al., 1998; Wang et al., 2016) emit fluorescence (Lai, Santangelo,  
69 Soressi, & Fantoni, 2007; Wang et al., 2010). Thus, the fluorescence properties of foods  
70 have a potential use for estimating the scavenging capacity.

71

72 Currently, there are few studies in which the potential use of absorption spectroscopic  
73 methods was investigated for estimating scavenging capacity (Lu, Ross, Powers, Aston,  
74 & Rasco, 2011). Infrared absorption spectra have been shown to predict well the  
75 scavenging capacity of fruit extracts (Lam, Proctor, Howard, & Cho, 2005), green tea  
76 (Zhang, Luypaert, Fernández Pierna, Xu, & Massart, 2004), and red wine (Versari,  
77 Parpinello, Scazzina, & Rio, 2010). However, when compared with FF, absorption  
78 spectroscopic method has lower sensitivity (Lakowicz, 2006). Prediction of scavenging  
79 capacity by FF has been investigated and validated in coffee and peppermint extracts  
80 (Orzel & Daszykowski, 2014) and tomato pastes (Orzel, Stanimirova, Czarnik-  
81 Matusiewicz, & Daszykowski, 2015); however, both studies employed ORAC, which  
82 represents only the scavenging capacity of particular radicals.

83

84 The objectives of this study were to investigate the possibility of using FF for  
85 estimating the scavenging capacities for various ROS. Peach, which is rich in functional  
86 components, was chosen as the target of this study. The scavenging capacities of peach  
87 extracts for four ROS (hydroxyl, alkoxy, superoxide radicals and singlet oxygen) were  
88 measured. **The scavenging capacities of phenolic compounds commonly found in peach**  
89 **were also measured to investigate their correlation with ROS scavenging capacity.** The

90 capability of FF to estimate the scavenging capacity for each ROS was examined using  
91 regression models and by an external validation method.

92

## 93 **2. Material and Methods**

### 94 *2.1 Samples*

95 Five fruits of each of the five peach cultivars (Asama Hakuto, Hakuho, Kawanakajima  
96 Hakuto, Natsukko, and Ougonto) were cultivated in Yamanashi Prefecture, Japan in  
97 2014. Aside from the yellow-skinned Ougonto, the remaining cultivars have the usual  
98 mixture of pink and yellow skin tone. Peach samples were immediately frozen in liquid  
99 nitrogen and were stored at  $-80\text{ }^{\circ}\text{C}$  until lyophilization. Frozen samples were  
100 lyophilized using a vacuum freeze drier (FDU-2110, EYELA, Tokyo, Japan) for 5 days.  
101 Freeze-dried whole fruit including skin and pulp was immediately ground in a  
102 mechanical mill (Waring blender 701 IHS, Osaka Chemical Co. Ltd., Osaka, Japan) and  
103 the resulting fine powders were stored at  $-30\text{ }^{\circ}\text{C}$  until performing the experiment.

104

### 105 *2.2 ROS scavenging capacity measurement of freeze-dried peach sample*

106 The scavenging capacities of the powdered freeze-dried peach samples for the hydroxyl,  
107 alkoxy, and superoxide radicals and singlet oxygen were measured by EPR spin  
108 trapping using an X-band EPR spectrometer (EMX-Plus, Bruker BioSpin, Karlsruhe,  
109 Germany) with 100 kHz field modulation. The measurement of scavenging capacity  
110 was performed based on MULTIS method (Oowada, Endo, Kameya, Shimmei, &  
111 Kotake, 2012).

112

113 The powdered freeze-dried peach samples (0.5 mg each) were dissolved in 1 mL of

114 ultrapure water (Wako Pure Chemical Industries, Osaka, Japan). All other chemicals for  
115 EPR measurement, unless stated, were purchased from Wako Pure Chemical Industries  
116 (Osaka, Japan) and were of highest grade. For the measurement of scavenging capacity  
117 for hydroxyl radicals, 50  $\mu$ L of the sample solution was mixed with 20  $\mu$ L of 5-(2,2-  
118 dimethyl-1, 3-propoxy cyclophosphoryl)-5-methyl-1-pyrroline N-oxide (CYPMPO)  
119 (Shidai Systems, Saitama, Japan) (5 mmol/L) as a spin trapping reagent, 50  $\mu$ L of  
120 hydrogen peroxide (200 mmol/L) as a precursor/sensitizer, 80  $\mu$ L of sodium phosphate  
121 buffer (PB) (100 mmol/L pH 7.4) and 30  $\mu$ L of diethylenetriaminepentaacetic acid  
122 (DTPA) (0.75 mmol/L). The radicals were generated by illuminating the reaction  
123 mixture with a light source in an EPR cavity, which was immediately followed by  
124 measurement of EPR spectra. The illumination was by UV light for 5 s. Mannitol was  
125 used as a standard chemical to build the calibration curve. For the measurement of  
126 scavenging capacity for alkoxy radicals (Kohri et al., 2009; Singh et al., 2009; Ukai,  
127 Kameya, Nakamura, & Shimoyama, 2009), 50  $\mu$ L of the sample solution was mixed  
128 with 20  $\mu$ L of CYPMPO (5 mmol/L) as a spin trapping reagent, and 50  $\mu$ L of AAPH (1  
129 mmol/L) as a precursor/sensitizer, 80  $\mu$ L of PB (100 mmol/L pH 7.4). The illumination  
130 was by UV light for 5 s. Trolox was used as a standard chemical to build the calibration  
131 curve. For the measurement of scavenging capacity for singlet oxygen, 30  $\mu$ L of the  
132 sample solution was mixed with 30  $\mu$ L of 2,2,6,6-Tetramethyl-4-piperidone  
133 hydrochloride (TMPD) (10 mmol/L) as a spin trapping reagent, 30  $\mu$ L of pterine (100  
134  $\mu$ mol/L) (Kohri et al., 2009; Oowada et al., 2012) as a precursor/sensitizer, 60  $\mu$ L of PB  
135 (100 mmol/L pH 7.4), 20  $\mu$ L of DTPA (1 mmol/L) and 130  $\mu$ L ultrapure water. The  
136 illumination was by UV light for 5 s. Glutathione (GSH) was used as a standard  
137 chemical to build the calibration curve. For the measurement of scavenging capacity for

138 superoxide radicals (Jiang, He, Pan, & Sun, 2010; Kim, Kirschenbaum, Rosenthal, &  
139 Riesz, 1993; Peng, Xiong, & Kong, 2009), 50  $\mu\text{L}$  of the sample solution was mixed with  
140 40  $\mu\text{L}$  of CYPMPO (20 mmol/L) as a spin trapping reagent, and 40  $\mu\text{L}$  of riboflavin (40  
141  $\mu\text{mol/L}$ ) as a precursor/sensitizer, 30  $\mu\text{L}$  of PB (100 mmol/L pH 7.4). The illumination  
142 was by visible light for 30 s.  $\alpha$ -lipoic acid was used as a standard chemical to build the  
143 calibration curve.

144

145 The typical spectrometer settings were as follows: resonance field, 3522.2 G; field  
146 modulation width, 1.00 G; and microwave power, 6 mW. EPR spectra were  
147 accumulated at room temperature. The UV light source for photolysis was a 200 W  
148 medium pressure mercury/xenon arc lamp (LC-8, Hamamatsu Photonics K.K.,  
149 Hamamatsu, Japan), in which UV–vis light was guided through a quartz light guide into  
150 the EPR sample cavity. G-533 band-pass filter (HOYA, Tokyo, Japan) was used. The  
151 illuminator was equipped with a computer controllable mechanical shutter, and the  
152 illumination period was set in the range of 0.1–100 s with a precision of 0.01 s. The UV  
153 illumination intensity was 2.78 mW/cm<sup>2</sup>.

154

### 155 *2.3 Quantification of total polyphenols*

156 The amount of total polyphenols in each powdered freeze-dried sample was measured  
157 by the Folin–Ciocalteu method. For the preparation of the samples, 80 mL of 50% ethyl  
158 alcohol was added to 0.2 g of the sample and mixed using ultrasonic sonicator (USM;  
159 AS ONE, Osaka, Japan). Ethyl alcohol (50%) was added to make 100 mL. The solution  
160 was centrifuged at 756  $\times g$  and 20  $^{\circ}\text{C}$  for 5 min (CR22G; Hitachi Koki, Tokyo, Japan)  
161 and the supernatant was obtained. One milliliter of the obtained supernatant was mixed

162 with 0.5 mL of Folin–Ciocalteu reagent (Sigma, USA; doubled dilution) and 5 mL of  
163 0.4 mol/L Na<sub>2</sub>CO<sub>3</sub>. After 30 min of incubation at 30 °C, the solution was cooled to  
164 room temperature and its absorbance was measured at 660 nm (Khalil et al., 2012). A  
165 calibration model was constructed using (+)-catechin at concentrations ranging from  
166 0.001 to 0.1 mg/mL. The total polyphenols concentration of the samples was expressed  
167 as catechin equivalents g/100 g freeze-dried sample weight.

168

#### 169 *2.4 Quantification of procyanidins by normal phase HPLC*

170 HPLC analysis of procyanidins was performed according to modified methods (Gu et  
171 al., 2002). Briefly, the freeze-dried peach powder (1 g) was extracted in a 15-mL screw-  
172 cap tube with 8 mL of extraction solvent (propanone/water/acetic acid (99.7%))  
173 (70:29.5:0.5, v/v/v) by shaking for 15 min under ambient conditions. Then, supernatant  
174 was collected by centrifugation (1500 ×g for 10 min) at 25 °C. Extraction procedures  
175 were repeated twice and supernatant was collected to a total volume of 25 mL. The  
176 collected supernatants were stored at -30 °C until HPLC analysis.

177

178 The quantitative analyses were performed using a Prominence HPLC system (Shimadzu  
179 Corporation, Kyoto, Japan) equipped with an RF-20AXS fluorescence detector  
180 (Shimadzu) with an Inertsil WP300 Diol (GL Science Inc., Tokyo, Japan) column (*i.d.*  
181 4.6 × 250 mm; 5 µm) at 30°C by the method of Obara et al. (2016). Mixtures of  
182 acetonitrile and acetic acid (mobile phase A, CH<sub>3</sub>CN/HOAc, 98:2, v/v) and methyl  
183 alcohol, H<sub>2</sub>O and acetic acid (mobile phase B, MeOH/H<sub>2</sub>O/HOAc, 95:3:2, v/v/v) were  
184 used as mobile phases. Elution was performed using a linear gradient of 0–7% B for 0–  
185 3.0 min, followed by a linear gradient of 7–30% B for 57.0 min. Subsequently, mobile

186 phase B contents were increased from 30% to 100% over 60.0–70.0 min. The mobile  
187 phase was subsequently returned to initial conditions (0% B) to re-equilibrate for 10.0  
188 min. Peach extracts from previously collected supernatants were filtrated through a 0.45  
189  $\mu\text{m}$  PTFE syringe filter and the sample injection volume was 5  $\mu\text{L}$ . The flow rate was  
190 set at 1.0 mL/min and the fluorescence of procyanidins was detected at excitation and  
191 emission wavelengths of 230 and 321 nm, respectively.

192

### 193 *2.5 Fluorescence fingerprint measurement*

194 One milligram of the powdered freeze-dried sample was mixed with one milliliter of  
195 Milli-Q water (Milli-Q Advantage; Merck Millipore, Germany), which was then  
196 pipetted into a quartz cuvette (FM20-SQ-3, GL Sciences, Tokyo). The fluorescence  
197 fingerprint measurement was conducted using a spectrofluorometer (FP-8500WRE;  
198 JASCO, Japan) with the front-face method (Sádecká & Tothová, 2007). The scanning  
199 speed was 20,000 nm/min. The excitation and emission ranges during the measurement  
200 were consequently set at 200–450 and 230–650 nm, respectively, with wavelength  
201 intervals of 5 nm. A photomultiplier voltage of 460 V and a response time of 20 ms  
202 were used. Other detailed settings were similar to previously reported work  
203 (Trivittayasil et al., 2016). Three replicates were performed for each sample.

204

### 205 *2.6 Prediction of ROS scavenging capacity by FF*

206 The capability of FF to estimate ROS scavenging capacity was investigated by partial  
207 least squares (PLS) regression analysis. The FF data served as a predictor whereas the  
208 regression targets were the scavenging capacities for all the target ROS. The FF data  
209 were prepared prior to the analysis by unfolding, which is the process that transforms

210 them into a data matrix, whose rows represent the samples and columns represent each  
211 excitation/emission wavelength condition (Smilde, Bro, & Geladi, 2005). The  
212 transformed FF data matrix contains a total of 75 samples (5 fruit samples  $\times$  5 cultivars  
213  $\times$  3 replicates) and 4335 variables (51 excitation wavelengths  $\times$  85 emission  
214 wavelengths). Mean centering as a preprocessing method was applied to the FF data  
215 matrix and regression targets. Three and two fruit samples of each cultivar were  
216 separated into calibration and validation groups, respectively. Cross-validation  
217 (Venetian blind with three splits and blindsize of three) was performed within the  
218 calibration group to determine the suitable number of latent variables. Three splits were  
219 used to ensure that each split has at least one fruit sample per cultivar and the blindsize  
220 of three was used to ensure that the replicates were placed in the same set to prevent  
221 overoptimistic estimation.

222

223 Multivariate analysis was conducted using R v3.2.2 software with the *EEM* v1.0.1 and  
224 *pls* v2.5-0 packages. *EEM* is a package developed by the authors for reading and  
225 preprocessing the fluorescence excitation–emission matrix. It was used to import and  
226 unfold raw three-dimensional data into the observation/variable matrix. PLS regression  
227 was carried out using *pls* package (Mevik & Wehrens, 2007). The number of latent  
228 variables for each PLS regression model was determined by visually locating a drop,  
229 known as the “knee”, in a scree plot of cross validation result (Henry, Park, &  
230 Spiegelman, 1999).

231

### 232 *2.7 Preparation of procyanidins and chlorogenic acid standards*

233 Procyanidin standards from monomer to heptamer were prepared using previously

234 modified methods (Shoji, Masumoto, Moriichi, Kanda, & Ohtake, 2006). Briefly,  
235 preparative chromatography of apple procyanidin standards was performed by  
236 conventional phase chromatography, and changes in the fluorescence of monomer–  
237 heptamer were recorded. Flavan-3ols/procyanidins of up to octamer lengths were eluted  
238 according to their degree of polymerization by preparative HPLC. Similar to previously  
239 reported procyanidins in cacao and chocolate (Hurst et al., 2009), clear relationships  
240 were observed with extremely high regression coefficients for standards covering a  
241 range of degree of polymerization ( $R^2 = 0.9987\text{--}0.9999$ ). The chlorogenic acid standard  
242 used here was purchased from Nacalai Tesque (Japan).

243

244 Procyanidins and chlorogenic acid were prepared with Milli-Q water to  $1 \times 10^{-5}$  mol/L and  
245  $1 \times 10^{-3}$  mol/L, respectively, and their fluorescence fingerprints were measured using the  
246 FP-8500WRE spectrofluorometer (JASCO, Japan).

247

#### 248 *2.8 Statistical analysis*

249 **The difference in the scavenging capacities for each of the four reactive oxygen species,**  
250 **total polyphenols and total procyanidins among cultivars was assessed using the analysis**  
251 **of variance (ANOVA) with post-hoc Tukey's honest significance test.** Principal  
252 component analysis (PCA) was also used understand the correlation between the  
253 variables. The data matrix with samples as rows and variables as columns was prepared.  
254 Autoscaling was used as a preprocessing method by centering columns to the zero mean  
255 and scaling to unit variance. The calculation was performed using R v3.2.2 software.

256

### 257 **3.Results and discussion**

258 *3.1 ROS scavenging capacity, polyphenols and procyanidins in peach extract*

259 The average scavenging capacities for the hydroxyl, alkoxy, superoxide radicals and  
260 singlet oxygen, total polyphenols and total procyanidins in peach cultivars measured by  
261 EPR spin trapping method are shown in Table 1. To comprehend the overall correlation  
262 between the scavenging capacities for various ROS, PCA was performed. The obtained  
263 PCA biplot is shown in Fig. 1. The first two principal components (PCs) could capture  
264 92.8% of the variances. The scavenging capacity for the hydroxyl radical was  
265 characterized by positive PC 2, whereas the remaining variables were characterized by  
266 positive PC 1. These results indicate that total polyphenols and procyanidins correlate  
267 with the scavenging capacities of all ROS except for the hydroxyl radical. The  
268 difference in the scavenging capacity for hydroxyl radical from those for other ROS  
269 agrees with the result previously reported (Kameya, Watanabe, Takano-Ishikawa, &  
270 Todoriki, 2014), in which the oxygen radical absorbance capacity of vegetables was  
271 found to correlate with the scavenging capacities for the alkoxy and superoxide radicals  
272 but not the hydroxyl radical. The reason for such phenomenon suggested in the above-  
273 mentioned literature was that the scavenging capacity of each ROS appears to depend  
274 on the specific components in each food type.

275

276 *3.2 Estimation of ROS scavenging capacity by FF*

277 All the five peach cultivars exhibit similar fluorescence patterns with three peaks at  
278 approximate excitation/emission wavelengths of 205/315, 225/320 and 280/310 nm  
279 (supplementary figure 1). There are many candidate components responsible for the  
280 peach FF profile, as peach contains various intrinsic fluorescent compounds such as  
281 hydroxycinnamates, procyanidins, flavonols and anthocyanins (Tomás-Barberán et al.,

282 2001). Two of the main peaks at excitation/emission wavelengths of 225/320 and  
283 280/310 nm correspond to epicatechin, which is prevalent in peach (Cheng & Crisosto,  
284 1995) and reported to exhibit fluorescence at 230/310 and 280/310 nm (Trivittayasil et  
285 al., 2015). A low-intensity peak at 330/440 nm could correspond to chlorogenic acid, as  
286 its fluorescence spectrum was reported to be approximately at 320/430 nm (Knee,  
287 1982).

288

289 The capability of FF to estimate ROS scavenging capacity was investigated using PLS  
290 regression with the regression targets being the scavenging capacity for each individual  
291 ROS. The results of PLS regression analysis are shown in Table 2. The scavenging  
292 capacities for the alkoxy and superoxide radicals could be well predicted by FF for an  
293 external validation dataset with the coefficients of determination of validation group  
294 ( $R^2P$ ) of 0.78 and 0.91, respectively. The prediction plots of alkoxy and superoxide  
295 radical scavenging capacity are shown in Fig. 2. The ratio of standard deviation to the  
296 root mean square error of prediction (RPD) was also calculated as a measure of the  
297 prediction power of a model. For the alkoxy radical, the RPD value is between 2.0 and  
298 2.5, indicating that the model can approximately quantify the response variable. On the  
299 other hand, the RPD value of the superoxide radical exceeds 2.5, suggesting that the  
300 model can predict the response very well. As the scavenging capacities for these two  
301 radicals were reported to highly correlate with ORAC values (Kameya et al., 2014), it  
302 can be noted that FF also has a potential use for estimating ORAC value.

303

304 The variables important to the prediction models of the superoxide and alkoxy radicals  
305 can be determined using the variable of importance projection (VIP). Variables with

306 VIP>1 are deemed to be important to the prediction model (Chong & Jun, 2005). The  
307 VIP plots of both the alkoxy and superoxide radicals are shown in Fig. 3, which appear  
308 very similar. This is due to the high correlation between the scavenging capacities of  
309 peach for the alkoxy and superoxide radicals (Pearson's  $r = 0.94$ ). The three main  
310 peaks of both VIP plots are approximately at excitation/emission wavelengths of  
311 210/310, 235/310 and 280/310 nm. Identification of the peaks is important to  
312 interpreting the model; however, it can be a difficult process because there are no  
313 existing libraries available for FF data. One solution is to compare the peak locations  
314 with the fluorescence spectra of standard compounds.

315

316 Comparison of these peaks with known fluorescence spectra from the literature  
317 (Trivittayasil et al., 2015) shows that the two peaks of the VIP plots correspond to  
318 epicatechin FF. This suggests that epicatechin plays an important role in scavenging the  
319 alkoxy and superoxide radicals. The fluorescence properties of components commonly  
320 found in peach such as procyanidins (Tomás-Barberán et al., 2001) and chlorogenic acid  
321 (Lavelli, Pompei, & Casadei, 2009) were measured. As procyanidins are oligomeric  
322 compounds formed from catechin and epicatechin, procyanidins of different degrees of  
323 polymerization were prepared (monomer–heptamer). FF data of the standard solutions  
324 of procyanidins and chlorogenic acid are shown in Fig. 4. The fluorescence patterns of  
325 procyanidins were found to be similar regardless of the degree of polymerization. There  
326 were two main peaks at excitation/emission wavelengths of approximately 220–  
327 240/310–320 nm and 280/310 nm. These two peaks were also observed in peach FF and  
328 were confirmed to correspond to procyanidins, including catechin and epicatechin as  
329 their monomers. Chlorogenic acid exhibits the fluorescence peak at the

330 excitation/emission wavelength of 340/450 nm, which can also be observed in peach  
331 FF. However, note that this fluorescence peak in peach FF could also be the isomeric  
332 counterparts of chlorogenic acid, which also emits fluorescence at the similar  
333 wavelengths (Tomás-Barberán et al., 2001).

334

335 As the fluorescence peak location of procyanidins has a VIP value higher than 1, it is  
336 considered to contribute to the good estimation of the scavenging capacities for the  
337 alkoxy and superoxide radicals by FF. This agrees with other reports in the literature  
338 that procyanidins correlate linearly with ORAC value (Adamson et al., 1999), which in  
339 turn correlates with the scavenging capacities for the alkoxy and superoxide radicals  
340 (Kameya et al., 2014). Procyanidins were also reported to be able to prevent lipid  
341 oxidation (Lotito et al., 2000). Goupy et al. (Goupy, Hugues, Boivin, & Amiot, 1999)  
342 found a very high correlation between the scavenging capacities of flavan-3-ols, in  
343 which procyanidins belong to, and the scavenging activity of barley measured by the  
344 DPPH method. Flavan-3-ols also showed the highest scavenging activities among all  
345 phenolic compounds tested and present in barley (Goupy et al., 1999) and apples (Lu &  
346 Yeap Foo, 2000).

347

348 In this study, as shown in table 2, the scavenging capacities for the hydroxyl radical and  
349 singlet oxygen cannot be estimated by FF, which could be due to the low correlation  
350 between them and polyphenols, whose fluorescence property is crucial to estimating the  
351 scavenging capacities for the other two ROS (alkoxy and superoxide radicals). The  
352 Pearson's correlation coefficients between the scavenging capacity for the hydroxyl  
353 radical and total polyphenols and procyanidins are very low: 0.078 and 0.075,

354 respectively. The Pearson's  $r$  between the scavenging capacity for singlet oxygen and  
355 total polyphenols and procyanidins are higher: 0.77 and 0.81, respectively. However,  
356 some studies indicated that there is no correlation between singlet oxygen quenching  
357 activity and polyphenols content (Sachindra, Airanthi, Hosokawa, & Miyashita, 2010).  
358 In addition, the small difference in the scavenging capacity for hydroxyl radical  
359 between cultivars could also be another reason for the low estimation of scavenging  
360 capacity for hydroxyl radical.

361

362 In conclusion, FF was shown to have a potential for well estimating the scavenging  
363 capacities of peach for the alkoxyl and superoxide radicals. The principal components  
364 that enable good estimation by FF for peach are suggested to be procyanidins, including  
365 their monomers and members of the flavan-3-ols family. Although this finding was able  
366 to successfully find the candidates for good estimation of scavenging capacity, for this  
367 technique to be useful in a practical setting, there are various issues that should be  
368 addressed. Some of the issues are the ability of FF to estimate the scavenging capacities  
369 of peach nondestructively, in which the difference in the scavenging capacities of peach  
370 skin and pulp should also be considered. In addition, as one of the limitations of this  
371 study is that only peaches from one harvest year were used, the good performance of FF  
372 in estimating the scavenging capacities of peach for some ROS should be further tested  
373 on more variability (more cultivars, year of production, etc.) of peach and other kinds of  
374 fruit.

375

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382

### 383 **5.Conflict of interest statement**

384 The authors certify that we have no affiliations with or involvement in any organization  
385 or entity with any financial interest, or non-financial interest in the subject matter or  
386 materials discussed in this manuscript.

387

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553

554 **Figure captions**

555 Fig. 1 PCA biplot of ROS (OH, hydroxyl radical; RO, alkoxy radical;  $^1\text{O}_2$ , singlet  
556 oxygen;  $\text{O}_2^-$ , superoxide radicals) scavenging capacities, total polyphenol and total  
557 procyanidins. The single letter on the score plot refers to the cultivars (A, Asaman  
558 Hakuto; H, Hakuho; K, Kawanakajima Hakuto; N, Natsukko; O, Ougonto)

559 Fig. 2 PLS regression prediction plots of alkoxy (RO) and superoxide ( $\text{O}_2^-$ ) radical  
560 scavenging capacity

561 Fig. 3 VIP score plots for alkoxy (RO) and superoxide ( $\text{O}_2^-$ ) radicals prediction models.  
562 The main peaks of both VIP score plots are at EX210/EM310, EX235/EM310 and  
563 EX280/EM310.

564 Fig. 4 Fluorescence fingerprints of standard solutions of phenolic compounds present in  
565 peach

566

567 Table 1 Average ROS scavenging capacities, total polyphenols and total procyanidins in  
 568 peach extract (mean  $\pm$  sd)

cultivar	scavenging capacity				total polyphenols	total procyanidins
	OH	RO	$^1\text{O}_2$	$\text{O}_2^-$	(g/100 g)	(mg/100 g)
Asama Hakuto	3.39 $\pm$ 0.63	0.33 $\pm$ 0.05 <sup>a</sup>	560.17 $\pm$ 234.59 <sup>a</sup>	1.99 $\pm$ 0.29 <sup>a</sup>	0.40 $\pm$ 0.11 <sup>a</sup>	56.17 $\pm$ 13.73 <sup>a</sup>
Hakuho	3.39 $\pm$ 0.41	0.46 $\pm$ 0.14 <sup>ab</sup>	1758.65 $\pm$ 363.45 <sup>b</sup>	3.27 $\pm$ 0.79 <sup>b</sup>	0.74 $\pm$ 0.13 <sup>b</sup>	28.43 $\pm$ 8.34 <sup>b</sup>
Kawanakajima Hakuto	3.53 $\pm$ 0.30	0.51 $\pm$ 0.08 <sup>bc</sup>	1489.63 $\pm$ 459.30 <sup>b</sup>	3.42 $\pm$ 0.68 <sup>b</sup>	0.68 $\pm$ 0.18 <sup>b</sup>	55.29 $\pm$ 16.96 <sup>b</sup>
Natsukko	3.21 $\pm$ 0.50	0.52 $\pm$ 0.06 <sup>bc</sup>	1518.48 $\pm$ 440.48 <sup>b</sup>	3.78 $\pm$ 0.54 <sup>bc</sup>	0.78 $\pm$ 0.12 <sup>bc</sup>	76.28 $\pm$ 13.53 <sup>bc</sup>
Ougonto	3.84 $\pm$ 0.34	0.65 $\pm$ 0.06 <sup>c</sup>	2052.82 $\pm$ 764.00 <sup>b</sup>	4.65 $\pm$ 0.56 <sup>c</sup>	1.02 $\pm$ 0.16 <sup>c</sup>	89.25 $\pm$ 13.69 <sup>c</sup>

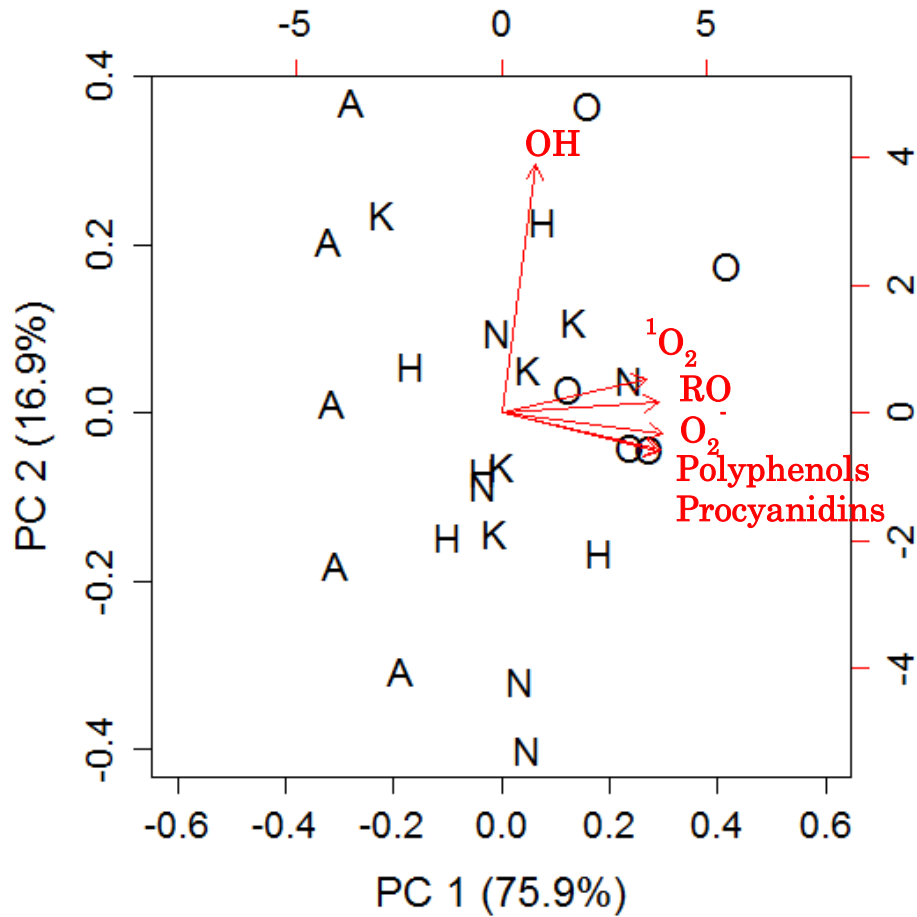
569 OH, hydroxyl radical (mmol mannitol eq./mL); RO, alkoxyl radical (mmol trolox  
 570 eq./mL);  $^1\text{O}_2$ , singlet oxygen (mmol GSH eq./mL);  $\text{O}_2^-$ , superoxide radical (mmol  $\alpha$ -  
 571 lipoic acid eq./mL); **Different superscript letters denote significant difference (P < 0.05)**  
 572

573 Table 2 PLS regression analysis result of scavenging capacity estimation of peach extract  
 574 for multiple ROS by fluorescence fingerprint

Objective variable <sup>a</sup>	ncomp <sup>b</sup>	Calibration		Validation		
		R <sup>2</sup> C	RMSEC	R <sup>2</sup> P	RMSEP	RPD
OH scavenging capacity	2	0.18	0.43	-0.80	0.43	0.76
RO scavenging capacity	2	0.86	0.05	0.78	0.06	2.18
<sup>1</sup> O <sub>2</sub> scavenging capacity	1	0.52	464.8	0.28	547.3	1.19
O <sub>2</sub> <sup>-</sup> scavenging capacity	2	0.94	0.24	0.91	0.31	3.41

575 <sup>a</sup> OH, hydroxyl radical (mmol mannitol eq./mL); RO, alkoxy radical (mmol trolox  
 576 eq./mL); <sup>1</sup>O<sub>2</sub>, singlet oxygen (mmol GSH eq./mL); O<sub>2</sub><sup>-</sup>, superoxide radical (mmol α-  
 577 lipoic acid eq./mL), <sup>b</sup> ncomp: number of latent variable components

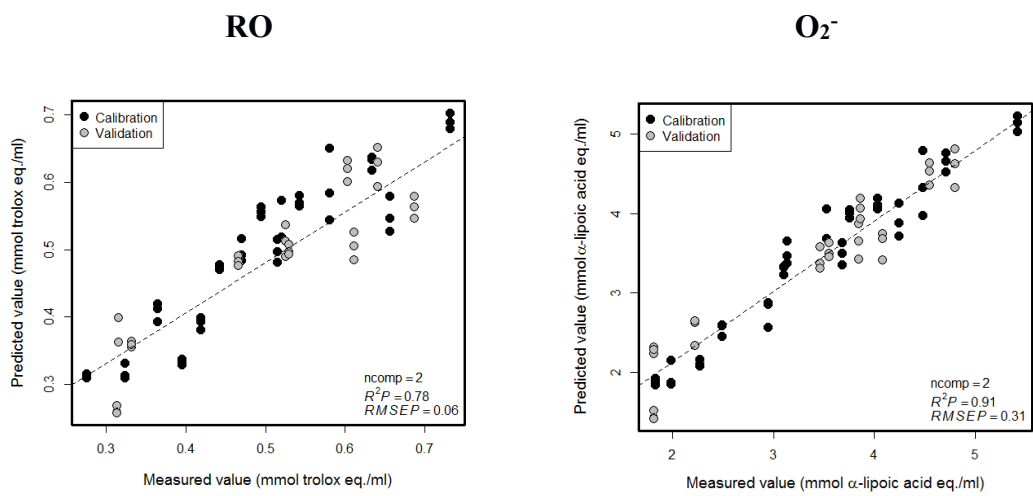
578



580

581 Fig. 1 PCA biplot of ROS (OH, hydroxyl radical; RO, alkoxyl radical; <sup>1</sup>O<sub>2</sub>, singlet  
 582 oxygen; O<sub>2</sub><sup>-</sup>, superoxide radicals) scavenging capacities, total polyphenols and total  
 583 procyanidins. The single letter on the score plot refers to the cultivars (A, Asaman  
 584 Hakuto; H, Hakuho; K, Kawanakajima Hakuto; N, Natsukko; O, Ougonto)

585

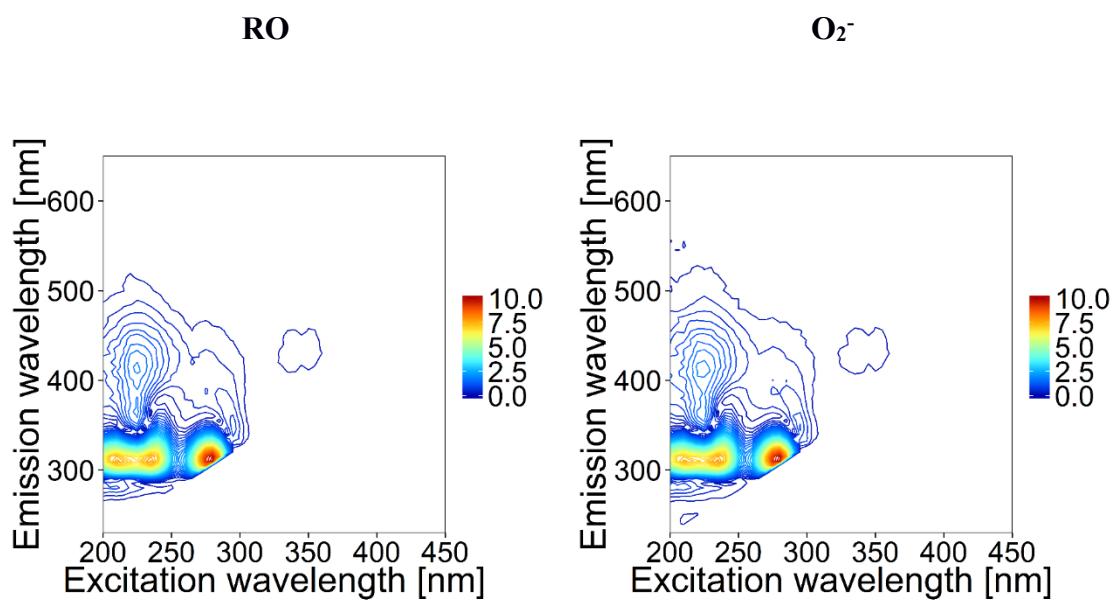


586

587 Fig. 2 PLS regression prediction plots of alkoxy (RO) and superoxide (O<sub>2</sub><sup>-</sup>) radical

588 scavenging capacity

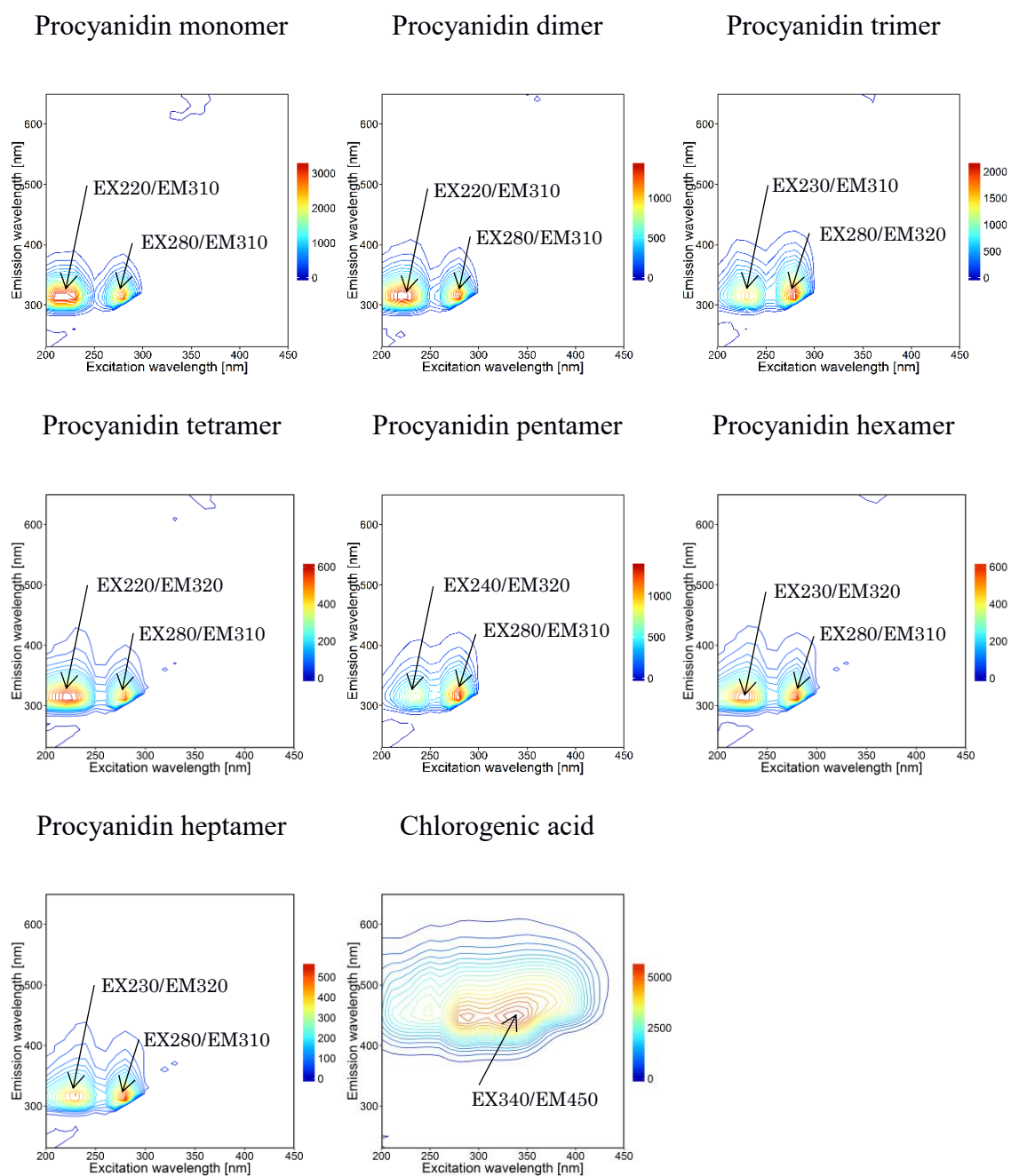
589



590

591 Fig. 3 VIP score plots for alkoxy (RO) and superoxide (O<sub>2</sub><sup>-</sup>) radical scavenging capacity  
 592 prediction models. The main peaks of both VIP score plots are at EX210/EM310,  
 593 EX235/EM310 and EX280/EM310.

594



595

596 Fig. 4 Fluorescence fingerprints of standard solutions of phenolic compounds present in

597 peach

598