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 α-lipoic acid eq./mL with a coefficient of determination of prediction (R2P) of 0.78 and 0.91, respectively.

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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, alkoxyl 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 alkoxyl 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

***Response to Reviewers**

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.

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 α-lipoic acid eq./mL with a coefficient of determination of prediction (R^2P) of 0.78 and 0.91, respectively.

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

1.Introduction

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

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

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

2.Material and Methods

2.1 Samples

Five fruits of each of the five peach cultivars (Asama Hakuto, Hakuho, Kawanakajima Hakuto, Natsukko, and Ougonto) were cultivated in Yamanashi Prefecture, Japan in 2014. Aside from the yellow-skinned Ougonto, the remaining cultivars have the usual mixture of pink and yellow skin tone. Peach samples were immediately frozen in liquid nitrogen and were stored at −80 °C until lyophilization. Frozen samples were lyophilized using a vacuum freeze drier (FDU-2110, EYELA, Tokyo, Japan) for 5 days. Freeze-dried whole fruit including skin and pulp was immediately ground in a mechanical mill (Waring blender 7011HS, Osaka Chemical Co. Ltd., Osaka, Japan) and the resulting fine powders were stored at -30 °C until performing the experiment.

2.2 ROS scavenging capacity measurement of freeze-dried peach sample

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

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

ultrapure water (Wako Pure Chemical Industries, Osaka, Japan). All other chemicals for EPR measurement, unless stated, were purchased from Wako Pure Chemical Industries (Osaka, Japan) and were of highest grade. For the measurement of scavenging capacity for hydroxyl radicals, 50 μL of the sample solution was mixed with 20 μL of 5-(2,2-dimethyl-1, 3-propoxy cyclophosphoryl)-5-methyl-1-pyrroline N-oxide (CYPMPO) (Shidai Systems, Saitama, Japan) (5 mmol/L) as a spin trapping reagent, 50 μL of hydrogen peroxide (200 mmol/L) as a precursor/sensitizer, 80 μL of sodium phosphate buffer (PB) (100 mmol/L pH 7.4) and 30 μL of diethylenetriaminepentaacetic acid (DTPA) (0.75 mmol/L). The radicals were generated by illuminating the reaction mixture with a light source in an EPR cavity, which was immediately followed by measurement of EPR spectra. The illumination was by UV light for 5 s. Mannitol was used as a standard chemical to build the calibration curve. For the measurement of scavenging capacity for alkoxyl radicals (Kohri et al., 2009; Singh et al., 2009; Ukai, Kameya, Nakamura, & Shimoyama, 2009), 50 μL of the sample solution was mixed with 20 μL of CYPMPO (5 mmol/L) as a spin trapping reagent, and 50 μL of AAPH (1 mmol/L) as a precursor/sensitizer, 80 μL of PB (100 mmol/L pH 7.4). The illumination was by UV light for 5 s. Trolox was used as a standard chemical to build the calibration curve. For the measurement of scavenging capacity for singlet oxygen, 30 μL of the sample solution was mixed with 30 μL of 2,2,6,6-Tetramethyl-4-piperidone hydrochloride (TMPD) (10 mmol/L) as a spin trapping reagent, 30 μL of pterine (100 μmol/L) (Kohri et al., 2009; Oowada et al., 2012) as a precursor/sensitizer, 60 μL of PB (100 mmol/L pH 7.4), 20 μL of DTPA (1 mmol/L) and 130 μL ultrapure water. The illumination was by UV light for 5 s. Glutathione (GSH) was used as a standard 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 μL of the sample solution was mixed with
140 40 μL of CYPMPO (20 mmol/L) as a spin trapping reagent, and 40 μL of riboflavin (40
141 μmol/L) as a precursor/sensitizer, 30 μL of PB (100 mmol/L pH 7.4). The illumination
142 was by visible light for 30 s. α-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^2.
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 ×*g* and 20 °C for 5 min (CR22G; Hitachi Koki, Tokyo, Japan)
161 and the supernatant was obtained. One milliliter of the obtained supernatant was mixed

with 0.5 mL of Folin–Ciocalteu reagent (Sigma, USA; doubled dilution) and 5 mL of 0.4 mol/L Na_2CO_3. After 30 min of incubation at 30 °C, the solution was cooled to room temperature and its absorbance was measured at 660 nm (Khalil et al., 2012). A calibration model was constructed using (+)-catechin at concentrations ranging from 0.001 to 0.1 mg/mL. The total polyphenols concentration of the samples was expressed as catechin equivalents g/100 g freeze-dried sample weight.

2.4 Quantification of procyanidins by normal phase HPLC

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

The quantitative analyses were performed using a Prominence HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with an RF-20AXS fluorescence detector (Shimadzu) with an Inertsil WP300 Diol (GL Science Inc., Tokyo, Japan) column (*i.d.* 4.6 × 250 mm; 5 µm) at 30°C by the method of Obara et al. (2016). Mixtures of acetonitrile and acetic acid (mobile phase A, CH_3CN/HOAc, 98:2, v/v) and methyl alcohol, H_2O and acetic acid (mobile phase B, MeOH/H_2O/HOAc, 95:3:2, v/v/v) were used as mobile phases. Elution was performed using a linear gradient of 0–7% B for 0–3.0 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 μm PTFE syringe filter and the sample injection volume was 5 μL. 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

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

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

2.7 Preparation of procyanidins and chlorogenic acid standards

Procyanidin standards from monomer to heptamer were prepared using previously

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

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

2.8 Statistical analysis

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

3.Results and discussion

3.1 ROS scavenging capacity, polyphenols and procyanidins in peach extract

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

3.2 Estimation of ROS scavenging capacity by FF

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

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

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

The variables important to the prediction models of the superoxide and alkoxyl radicals 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 alkoxyl 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 alkoxyl 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 alkoxyl 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 alkoxyl 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 alkoxyl 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 (alkoxyl 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
376 **4.Acknowledgements**
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and Fisheries under the "Development of non-destructive technology evaluating various qualities of agricultural products" project and by Japan Society for the Promotion of Science. We would like to thank Yasuyo Sekiyama and Satoru Tomita of Food Research Institute for providing the chlorogenic acid standard.

5.Conflict of interest statement

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

6.References

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554 **Figure captions**

555 Fig. 1 PCA biplot of ROS (OH, hydroxyl radical; RO, alkoxyl radical; $^1O_2$, singlet
556 oxygen; 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 alkoxyl (RO) and superoxide (O_2^-) radical
560 scavenging capacity

561 Fig. 3 VIP score plots for alkoxyl (RO) and superoxide (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 ± sd)

cultivar	scavenging capacity				total polyphenols	total procyanidins
	OH	RO	$^1O_2$	O_2^-	(g/100 g)	(mg/100 g)
Asama Hakuto	3.39 ± 0.63	0.33 ± 0.05 ^{a}	560.17 ± 234.59 ^{a}	1.99 ± 0.29 ^{a}	0.40 ± 0.11 ^{a}	56.17 ± 13.73 ^{a}
Hakuho	3.39 ± 0.41	0.46 ± 0.14 ^{ab}	1758.65 ± 363.45 ^{b}	3.27 ± 0.79 ^{b}	0.74 ± 0.13 ^{b}	28.43 ± 8.34 ^{b}
Kawanakajima Hakuto	3.53 ± 0.30	0.51 ± 0.08 ^{bc}	1489.63 ± 459.30 ^{b}	3.42 ± 0.68 ^{b}	0.68 ± 0.18 ^{b}	55.29 ± 16.96 ^{b}
Natsukko	3.21 ± 0.50	0.52 ± 0.06 ^{bc}	1518.48 ± 440.48 ^{b}	3.78 ± 0.54 ^{bc}	0.78 ± 0.12 ^{bc}	76.28 ± 13.53 ^{bc}
Ougonto	3.84 ± 0.34	0.65 ± 0.06 ^{c}	2052.82 ± 764.00 ^{b}	4.65 ± 0.56 ^{c}	1.02 ± 0.16 ^{c}	89.25 ± 13.69 ^{c}

569 OH, hydroxyl radical (mmol mannitol eq./mL); RO, alkoxyl radical (mmol trolox

570 eq./mL); $^1O_2$, singlet oxygen (mmol GSH eq./mL); O_2^-, superoxide radical (mmol α-

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 [a]	ncomp [b]	Calibration		Validation		
		R^2C	RMSEC	R^2P	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
$^1O_2$ scavenging capacity	1	0.52	464.8	0.28	547.3	1.19
O_2^- scavenging capacity	2	0.94	0.24	0.91	0.31	3.41

575 [a] OH, hydroxyl radical (mmol mannitol eq./mL); RO, alkoxyl radical (mmol trolox
576 eq./mL); $^1O_2$, singlet oxygen (mmol GSH eq./mL); O_2^-, superoxide radical (mmol α-
577 lipoic acid eq./mL), [b] ncomp: number of latent variable components
578

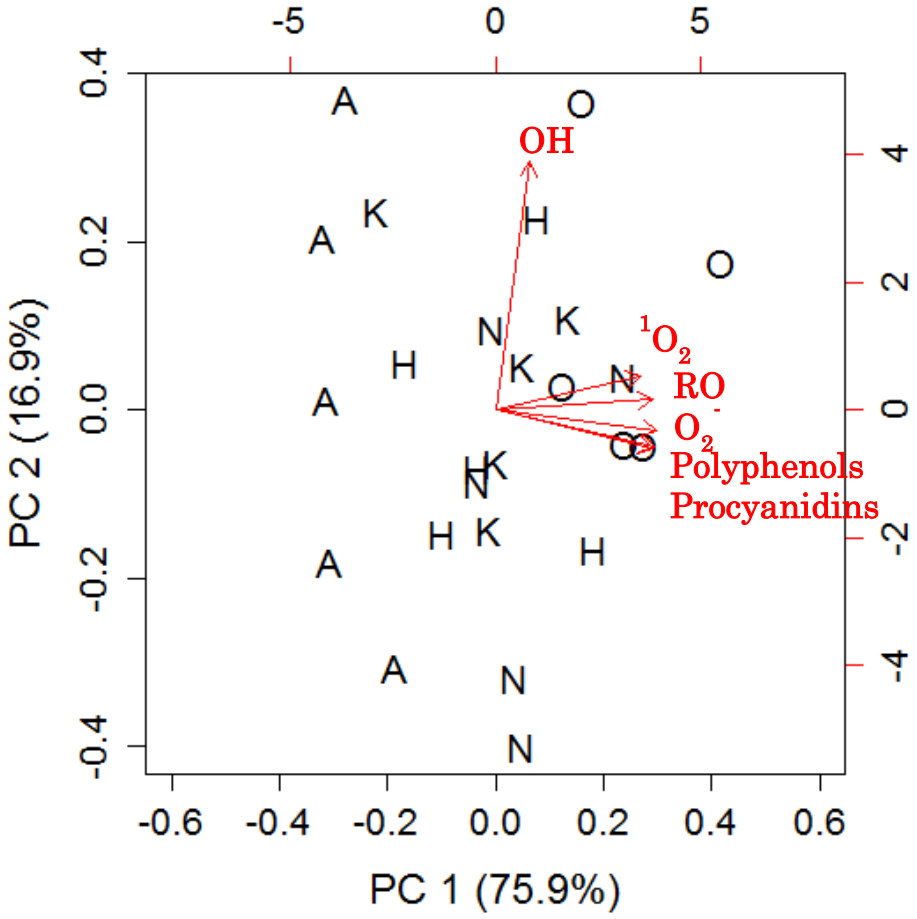
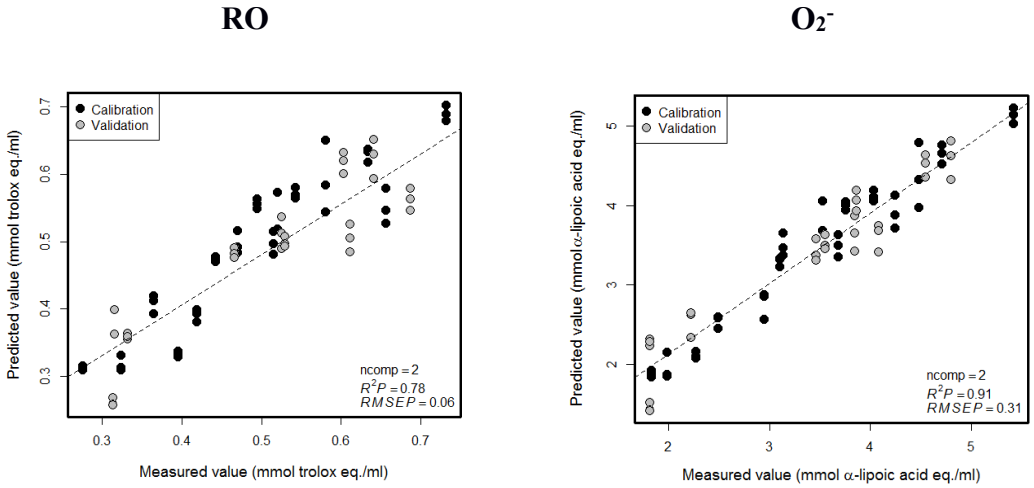


Fig. 1 PCA biplot of ROS (OH, hydroxyl radical; RO, alkoxyl radical; $^1O_2$, singlet oxygen; O_2^-, superoxide radicals) scavenging capacities, total polyphenols and total procyanidins. The single letter on the score plot refers to the cultivars (A, Asaman Hakuto; H, Hakuho; K, Kawanakajima Hakuto; N, Natsukko; O, Ougonto)

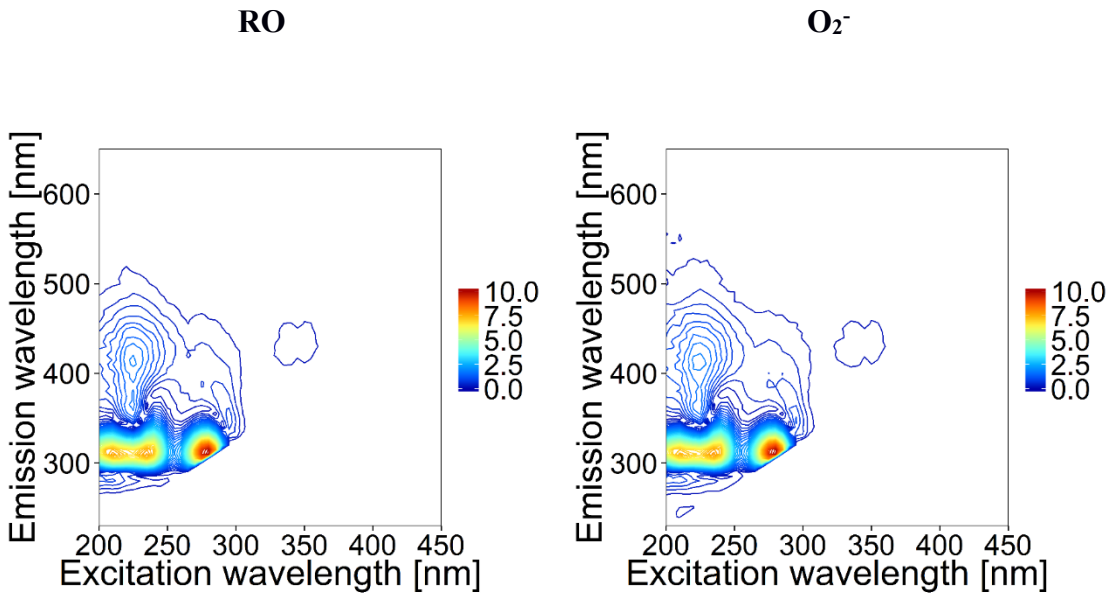


586

587 Fig. 2 PLS regression prediction plots of alkoxyl (RO) and superoxide (O_2^-) radical

588 scavenging capacity

589



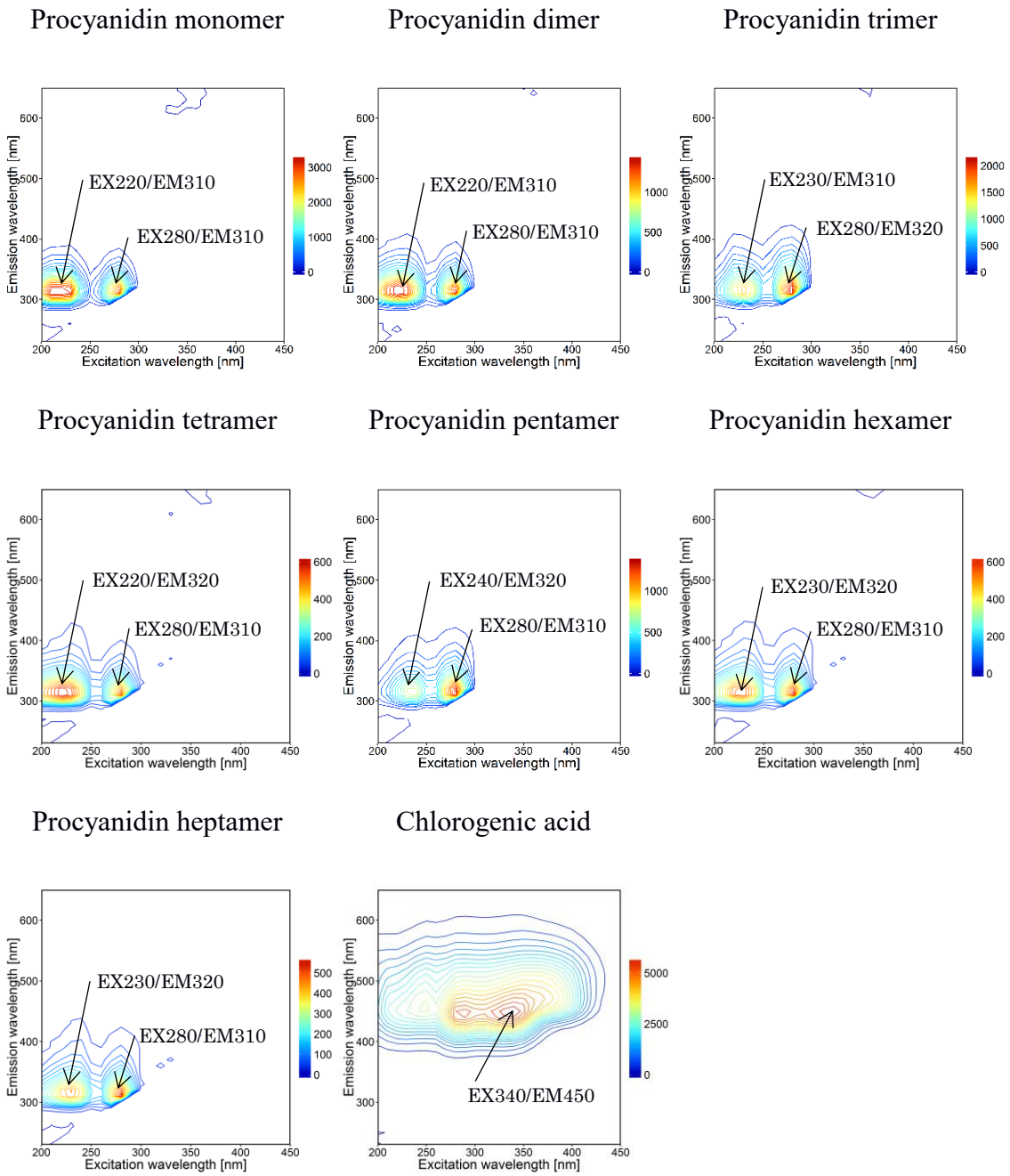
590

591 Fig. 3 VIP score plots for alkoxyl (RO) and superoxide (O_2^-) 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



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

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