Quality changes in perilla seed powder related to storage duration and temperature

Yejin Son¹ · Kyo-Yeon Lee² · Suyeon Gu¹ · Ji Yeong Park¹ · Sung-Gil Choi² · Hyun-Jin Kim^{1,2}

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Abstract Perilla seed powder (PSP) was stored at 25 °C, 35 °C, and 45 °C for 8 weeks. Changes in the metabolite profiles of the powders, including fatty acids, were monitored. Correlations between these changes and quality parameters, including lipid oxidation, color, and antioxidant activity, were analyzed to evaluate the effects of storage duration and temperature on PSP quality. Acid values increased significantly with the duration of storage, but not with temperature. Multivariate statistical analysis was performed to identify differences among the metabolite profiles. The PSP sample stored for 1 week at 45 °C and all samples stored at 25 °C and 35 °C were grouped separately from the control and samples stored at 45 °C for more than 4 weeks. Among the many metabolites associated with these differences, lysophosphatidylethanolamines, tocopherol, sitosterol, tryptophan, 12-hydroxyjasmonic acid glucoside, and maltose correlated negatively with quality parameters with the exception of L* and antioxidant activity. Luteolin, apigenin, luteolin 4'-methyl ester, citric acid, isocitric acid, 9(S)-HPODE, and 3,5-octadien-2-one correlated positively with quality. Although the quantities of some antioxidants and lipids decreased during

storage, the results suggested that the quality of PSP samples stored at 25 °C, 35 °C, and 45 °C for 8 weeks was acceptable. This was because lipid oxidation promoted by the storage environment was limited by antioxidants in the samples. These metabolites could be useful for monitoring changes in PSP quality.

Keywords Antioxidant · Lipid oxidation · Metabolomics · Perilla seed powder · Storage

Introduction

Perilla (*Perilla frutescens*), an annual plant in the mint family (Lamiaceae), is widely cultivated in Asia as a traditional medicine and food supplement (Omer et al. 1998). Perilla seed is primarily used to produce edible oil, because the oil is a rich source of unsaturated fatty acids that confer various health benefits. These include oleic acid, linoleic acid, and linolenic acid (Guan et al. 2014). Several clinical and animal studies have shown that compounds in perilla oil have diverse phytopharmacological effects. Antitumor, anti-inflammatory, hypotensive, anti-atherosclerotic, and antidepressant activity has been reported, as well as the mitigation of anaphylactic shock (Watanabe et al. 1994; Ezaki et al. 1999; Yu et al. 2017). Perilla seed powder (PSP) contains the same fatty acids found in perilla oil, although PSP is used as a seasoning due to its unique taste and flavor. The nutritional quality of PSP can be diminished by suboptimal storage conditions, because unsaturated fatty acids in the powder are readily oxidized. Temperature, humidity, light, and microbial contamination are storage factors commonly associated with changes in the nutritional value of grains, vegetables, fruits, and processed products that contain them (Gil et al. 2006).

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✉ Hyun-Jin Kim
hyunjkim@gnu.ac.kr

¹ Division of Applied Life Sciences (BK21 Plus), Gyeongsang National University, 501 Jinjudaero, Jinju, Gyeongsang, Republic of Korea

² Department of Food Science and Technology, and Institute of Agriculture and Life Science, Gyeongsang National University, 501 Jinjudaero, Jinju, Gyeongsang, Republic of Korea

However, scientific evidence of a relationship between changes in PSP nutritional quality and storage conditions is lacking.

Various omics technologies have been employed to gain a better understanding of nutritional changes in edible plants and other foods due to storage conditions (Liao et al. 2018). Tools used for metabolomic investigation include nuclear magnetic resonance (NMR) spectroscopy, liquid chromatography coupled with mass spectrometry (LC/MS), gas chromatography/mass spectrometry (GC/MS), and multivariate statistical analysis. These techniques have been applied to detect changes in the nutritional quality of processed foods and living organisms (Dunn et al. 2005; Wishart et al. 2001; Wishart 2008). The metabolite profiles of edamame (Sugimoto et al. 2010), red wine (Arapitsas et al. 2016), lamb (Reis et al. 2016), kimchi (Jeong et al. 2013), and rice (Lee et al. 2018) have been studied under various storage conditions. However, no such study has been performed with PSP. In the present work, LC/MS was used to monitor changes in the metabolite profiles of PSP samples stored for 8 weeks at 25 °C, 35 °C, or 45 °C. The fatty acid profiles of the samples were analyzed by GC/MS. Correlations between the observed changes and quality parameters were also investigated.

Materials and methods

Perilla seed powder preparation and storage

Perilla seeds from the *Dayu* cultivar harvested in 2017 were kindly supplied by Chungbuk Agricultural Research and Extension Service of Korea. The seeds were washed and dried at 40 °C by a dry oven (JS-150SP, Jinscience, Gimcheon, Korea) until their moisture content reached 2%. The dried seeds were powdered in a vacuum-nitrogen displacement grinding device (Kim et al. 2017). The powders were placed on aluminum dishes in desiccators maintained at 66% relative humidity (RH) using saturated cobalt chloride solution. Each desiccator with normal air composition was stored in the dark for 8 weeks at 25 °C, 35 °C, or 45 °C. Samples were collected from the powders at 1, 4, and 8 weeks. Freshly ground PSP that did not undergo storage served as a control.

Water activity, appearance, and lipid oxidation values

The water activity was measured using a water activity meter (AQS-2, Nagy mess system, Gäufelden, Germany). Free fatty acids present in PSP lipids and their oxidation were determined using the acid value (AV) and peroxide value (POV), respectively, according to the method of the

Association of Official Analytical Chemists (1987). AV and POV were reported in mg KOH/g and meq/kg, respectively. Color changes in the PSPs were measured with a CR 301 colorimeter (Minolta Co., Osaka, Japan), which was calibrated with standard plates (lightness (L^*) = 96.94; a^* = 0.66, and b^* = 0.51) using the illuminant D65 and the 10° standard observer.

Determination of total phenolic content and antioxidant activity

The total phenolic compounds (TPC) in each PSP sample were analyzed using Folin–Ciocalteu's reagent (FCR) according to the method of Singleton and Rossi (1965). Phenolic compounds were extracted from PSP samples using 80% methanol. The methanolic extract (50 μ L) was mixed with 450 μ L distilled water and 250 μ L 2 N FCR. The mixture was added to 1.25 mL 20% Na_2CO_3 and incubated at 25 °C for 20 min. After centrifugation, the absorbance of the supernatant was measured at 735 nm using a UV–VIS spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan), and a standard curve (R^2 = 0.966) was prepared with gallic acid (GA). The absorbance was converted to phenolic content in units of milligrams GA equivalent (GAE) per gram of sample. The antioxidant activity of PSP, which was extracted using 80% methanol, was determined with the 2,2-diphenyl-1-picrylhydrazyl (DPPH·) free radical scavenging assay (Yamaguchi et al. 1998), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay (Re et al. 1999), and metal chelating assay (Dinis et al. 1994) with slight modification. For DPPH· free radical scavenging assay, a reaction mixture was prepared with 0.5 mL of a diluted PSP sample and 0.5 mL of 0.3 mM methanolic DPPH· solution. The mixture was shaken and incubated in darkness at room temperature for 20 min. After incubation, the absorbance of the reaction mixture was measured at 517 nm using a UV–VIS spectrophotometer, and DPPH· free radical scavenging activity was calculated. A standard curve (R^2 = 0.976) was prepared with trolox, and the DPPH· free radical scavenging activity was reported as mg trolox equivalent (TE) per gram of sample. For ABTS· radical solution generated by blending 0.7 mM ABTS stock solution with 2.45 mM potassium phosphate-buffered solution (PBS, pH 7.4) was mixed with 10 μ L of the sample extract. After 7 min, the absorbance of the sample mixture was measured at 734 nm using a UV–Vis spectrophotometer. A standard curve (R^2 = 0.994) was prepared with trolox and the ABTS· radical scavenging activity was reported as mg TE per gram of sample. For metal chelating assay, 0.2 mL of the sample extract was mixed with 0.1 mM FeSO_4 and 0.25 mM ferrozine. After incubating at room temperature for 10 min, the absorbance

of the mixture was measured at 562 nm using a UV–Vis spectrophotometer. A standard curve ($R^2 = 0.984$) was prepared with ethylenediaminetetraacetic acid (EDTA), and the metal chelating activity was reported as mg EDTA per gram of sample.

Analysis of fatty acids, volatile compounds, and phytosterols

The fatty acid composition, volatile compounds, and phytosterol content of the PSP samples were analyzed by GC/MS (Lee et al. 2016). Fatty acids were extracted from PSP with 2 mL 0.5 M KOH in methanol at 90 °C for 15 min. BF_3 in methanol (1 mL) was added to each extract (0.5 mL) to methylate the fatty acids. The mixtures were incubated at 90 °C for 2 min, then cooled. Saturated NaCl solution (2.5 mL) and 2 mL hexane containing 5 α -cholestane as an internal standard (IS) were added to each sample. After centrifugation, 500 μL of the hexane layer was removed. N,O -bis(trimethylsilyl)trifluoroacetamide (BSTFA, 150 μL) was added to each sample for derivatization. Derivatization was carried out at 70 °C for 20 min.

Volatile compounds were analyzed using solid phase microextraction (SPME)-GC/MS. PSP samples (0.1 g) were mixed with 0.5 mL of distilled water contained 2-methylpentanol as an internal standard in a sealed vial with a septum cap. The vial was equilibrated at 65 °C for 10 min. After heating, a SPME fiber (50/30 μm , DVB/CAR/PDMS Stableflex, Supleco Inc., Bellefonte, PA, USA) was inserted into the vial cap to absorb volatile compounds in the head space at 65 °C for 15 min. The absorbed volatile compounds were then analyzed using GC/MS.

For phytosterol analysis, PSP samples (50 mg) were extracted with MTBE (1 mL) for the phytosterol analysis. The derivatized fatty acids and the volatile compounds were analyzed on a GC-2010 plus gas chromatograph (Shimadzu, Tokyo, Japan) equipped with a DB-Wax column (30 m $\times$ 0.25 mm, 0.25 μm , Agilent, U.S.A.), while a DB-5 ms capillary column (30 m $\times$ 0.25 mm, 0.25 μm , Agilent J&W) was used to separate phytosterols. The derivatized fatty acids and phytosterol extract were injected with a 1:50 split, while the volatile compounds were injected with splitless. Helium was used as the carrier gas at a flow rate of 1 mL/min, and the injection temperature was set at 250 °C. For the fatty acid analysis, the oven temperature was held at 210 °C for 5 min, then increased to 250 °C at 4 °C/min. For the volatile compound analysis, the oven temperature was held at 40 °C for 3 min, increased to 90 °C at 5 °C/min, and then increased to 230 °C at 19 °C/min. For the phytosterol analysis, the oven was held at 60 °C for 2 min, then heated to 320 °C at 20 °C/min. The GC column effluent was detected using a

TQ 8030 mass spectrometer (Shimadzu) with the electron ionization mode at 70 eV. The temperatures of the MS source and interface were set to 230 °C and 250 °C, respectively. The mass spectra were collected in full scan mode from m/z 45 to m/z 550 with a scan time of 0.3 s and a scan speed of 2000 amu/s. All MS data were normalized to the IS. Fatty acids were identified by their retention indices (RIs), which were normalized to a series of *n*-alkanes (C8–C40), the Wiley and NIST mass spectral databases, and authentic standards.

Metabolite analysis by UPLC-Q-TOF MS

Metabolites extracted from the PSP samples with 80% methanol containing IS (abscisic acid) were analyzed with ultra-high performance liquid chromatography-quadrupole time-of-flight (UPLC-Q-TOF) MS in negative ion mode. The extracted samples were separated on an Acquity UPLC BEH C18 column (2.1 mm $\times$ 100 mm, 1.7 μm , Waters Corp. U.S.A.) equilibrated with H_2O :acetonitrile (ACN) containing 0.1% formic acid. Elution was carried out at a flow rate of 0.35 mL/min for 12 min. A Xevo G2-S Q-TOF MS (Waters Corp., Milford, MA) was used for detection. The voltages of the capillary and sampling cones were set at 3 kV and 30 V, respectively. The LockSprayTM electrospray ionization source and quadrupole were operated at 200 °C and 100 °C, respectively. Leucine-enkephalin ($[\text{M}-\text{H}]^- = 554.2615$ Da) was used as a reference solution for exact mass measurement and introduced via the LockSpray source at a flow rate of 20 $\mu\text{L}/\text{min}$. Mass spectral data were collected from m/z 100 to 1000 with a scan time of 0.2 s. Collision energy was ramped from 10 to 40 eV for collection of MS/MS data. For quality control, a mixture of all PSP samples was injected after every 10 samples. Metabolite peaks were recorded over 1 s using the peak width at 5% height with an intensity threshold of 10,000, peak-to-peak baseline noise of 1, and noise elimination set to 6. Peaks were aligned within a 0.05 Da mass window and a retention time window of 0.2 min using MarkerLynx software (Waters). All mass spectra were normalized to the IS. The metabolites were identified using ChemSpider (www.chemspider.com), a human metabolome database (www.hmdb.ca), the METLIN database (metlin.scripps.edu), and authentic standards.

Statistical analysis

The processed fatty acid and metabolite data sets were evaluated by multivariate statistical analysis with SIMCA-P+ version 12.0.1 (Umetrics, Umeå, Sweden). Partial least squares discriminant analysis (PLS-DA) was performed to visualize the differences among samples and to identify the metabolites that contributed to the differences. The quality

Fig. 1 Time course of color changes, acid values, peroxide values, and antioxidant activities in PSP samples stored at 25 °C, 35 °C, and 45 °C for 0, 1, 4, and 8 weeks. Different letters appearing within the same week indicate significant differences at $p < 0.05$

of the PLS-DA models was evaluated by their goodness of fit (R^2X and R^2Y) and predictive ability (Q^2Y), and they were cross-validated with a permutation test ($n = 200$). One-way analysis of variance (ANOVA) with Duncan's test ($p < 0.05$) was performed using the SPSS 17.0 package (SPSS Inc., U.S.A.) to identify significant differences among the sample groups. The relative abundance of identified metabolites and the correlation between metabolites and other quality parameters were visualized with a heat map generated in R corplot. ANOVA with Duncan's test ($p < 0.05$) was performed with all of the color, antioxidant activity, TPC, AV, and POV data.

Results and discussion

Water activity, appearance, lipid oxidation, and antioxidant activity

The water activity of PSP samples stored in desiccators maintained at 66% RH was about 0.64 and the value was not changed by storage duration and temperature. The appearance of PSP samples stored at 25 °C and 35 °C

remained nearly unchanged after 8 weeks of storage. However, as the storage duration increased at 45 °C, the sample color became lighter. The PSP sample stored at 45 °C was light yellow in color after 4 days of storage (Supplementary Fig. 1a). Changes in the appearance of the samples were evaluated with additional color parameters, each of which varied with increasing storage duration and temperature (Fig. 1a). The lightness (L^*) of the PSP samples stored at 35 °C and 45 °C increased from an initial value of 43.38 to 47.19 by week eight, but L^* of the sample stored at 25 °C for 8 weeks was slightly lower at 45.99. The a^* and b^* values of the PSP samples stored at 45 °C were 6.22 and 20.23, respectively. The a^* and b^* values of these samples increased by 88% and 60%, respectively, relative to a^* (3.3) and b^* (12.6) of the control. The a^* values of the PSP samples stored at 25 °C and 35 °C did not change significantly with the duration of storage, while their b^* values increased by 20% after 8 weeks. There was no significant difference between the samples stored at 25 °C and 35 °C.

In addition to changes in appearance, longer storage periods and higher temperature promoted lipid oxidation in the PSP samples. The AV of the PSP samples stored for

Fig. 2 **a** Representative chromatograms of global metabolite profiles in PSP, **b** fatty acid profiles, **c** phytosterols. Metabolites extracted in 80% methanol were analyzed by UPLC-Q-TOF MS, while fatty acids and phytosterols were analyzed by GC-MS. Identified metabolites, including fatty acids and phytosterols, were numbered: 1, maltose; 2, citric acid; 3, isocitric acid; 4, tryptophan; 5, 12-hydroxyjasmonic acid glucoside; 6, rosmarinyl glucoside; 7, viscumneoside VI; 8,

rosmarinic acid; 9, eupatorin; 10, luteolin; 11, apigenin; 12, luteolin 4'-methyl ether; 13, 9(S)-HPODE; 14, asiatic acid; 15, LPE(C20:3); 16, PI(C16:0); 17, LPE(C18:2); 18, hydroxylinolenic acid; 19, LPE(C18:0); 20, LPE(C16:0); 21, palmitic acid; 22, stearic acid; 23, oleic acid; 24, linoleic acid; 25, linolenic acid; 26, tocopherol; and 27, sitosterol

8 weeks was 6 mg KOH/g, which was five times higher than that of the control (1.2 mg KOH/g). AV varied little among the stored PSP samples, although the AVs of samples stored at 25 °C for more than 4 weeks were slightly lower than those of the other samples (Fig. 1b). The initial POVs of the PSP samples were ~ 6.0 meq/kg, and the magnitude of changes in POV was smaller than that observed in AV. The POVs of the PSP samples stored at 35 °C and 45 °C increased with the duration of storage. After 8 weeks, their POVs increased to 9.3 meq/kg (35 °C) and 12.3 meq/kg (45 °C), which were respectively 1.6- and twofold higher than the POV of the control (Fig. 1c). The POVs of samples stored at 25 °C remained unchanged for the first week of storage; but after 4 weeks, the POV at 25 °C was similar to the POV of samples stored at 35 °C. These results indicated that environmental factors related to storage promoted the hydrolysis of perilla oil, leading to the accumulation of free fatty acids as indicated by AV.

However, lipid oxidation in PSP did not progress far enough within 8 weeks to induce rancidity. This was attributed to the many antioxidant compounds in the samples (Chapman et al. 2009). In all PSP samples with an initial DPPH $\cdot$ free radical scavenging activity of 43 mg TE/g and metal chelating activity of 77 mg EDTA/g, antioxidant activity decreased significantly with storage duration and temperature (Fig. 1d). DPPH $\cdot$ free radical scavenging activities of samples stored at 25 °C, 35 °C, and 45 °C for 8 weeks were 16 mg TE/g, 22 mg TE/g, and 11 mg TE/g, respectively, although the decreased activities at 1 week increased at 4 weeks, while their metal chelating activities at 8 weeks were 60 mg EDTA/g, 29 mg EDTA/g, and 21 mg EDTA/g, respectively. However, ABTS $\cdot$ free radical scavenging activities of samples were not changed by storage duration and temperature. Similar results were reported that lipid oxidation in many vegetable oils was prevented by treatment of antioxidants (Abdelazim et al.

Fig. 3 **a** PLS-DA score plots of PSP samples stored at different temperatures for 0, 1, 4, and 8 weeks. **b** PLS-DA score plots visualized separately according to storage duration and **c** temperature.

2013; Suja et al. 2004). The POVs of PSP samples stored for 8 weeks were higher than those of refined oils (Hashempour-Baltork et al. 2018), but lower than those of crude black cumin oil, niger seed oil, corn oil, and milled grains (Ramadan and Mörsel 2004; Winkler-Moser and Breyer 2011). A POV of 10–15 meq/kg or less is acceptable according to the Codex Alimentarius (2001). Thus, PSP stored for 8 weeks with a POV of 12 meq/kg or less could be considered fresh.

Change of PSP fatty acid and metabolite profiles with temperature and duration of storage

To investigate the relationship between storage conditions and the nutritional quality of PSP, fatty acids and metabolites were analyzed by GC/MS and UPLC-Q-TOF MS, respectively, and the MS data underwent statistical analysis by PLS-DA. The metabolite profiles included sugars, amino acids, phenolic compounds, lipids, phytosterols, and volatile compounds (Fig. 2a, and Supplementary Fig. 2). The fatty acid profiles included palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid (Fig. 2b). PLS-DA scores were plotted to visualize the differences among sample groups (Fig. 3) with

The qualification of the PLS-DA models were qualified using R^2X , R^2Y , Q^2 , and p values and cross-validated with a permutation test ($n = 200$) (Supplementary Fig. 2)

acceptable quality parameters. The R^2X , R^2Y , and Q^2 values indicated that goodness of fit was acceptable for all of the PLS-DA models. $R^2 < 0.33$ for all samples, while $R^2 < 0.58$ for temperature and storage duration. The predictive ability, Q^2 , was less than 0.38 for all samples, while $Q^2 < 0.78$ for temperature and storage duration. The p values were less than 0.05 with the exception of the 1-week model. Cross-validation with a permutation test indicated that the R^2 and Q^2 intercept values were < 0.8 and < -0.05 , respectively, with the exception of the 1-week model (Supplementary Fig. 3). These values indicated that all of the PLS-DA models were adequate, with the exception of the 1-week model. However, some of the PLS-DA models showed slightly higher R^2 intercepts of cross-validation. All samples stored at 25 °C and 35 °C and the 45 °C sample stored for 1 week were not distinguished on the PLS-DA score plot. However, clear separation between these groups and PSP samples stored at 45 °C for more than 4 weeks was indicated by the first two components. All sample groups were also separated from the control (Fig. 3a).

To investigate the individual effects of storage temperature or duration on the fatty acid and metabolite profiles of the PSP samples, all sample groups were compared

Table 1 Identification of metabolites contributing to the separation among sample groups on the PLS-DA scores plots of the dataset analyzed by GC/MS and LC/MS

NO	Metabolites	VIP ^a	<i>p</i> value ^b
1	Maltose	0.77	3.33E–07
2	Citric acid	0.93	5.14E–10
3	Isocitric acid	1.37	1.67E–08
4	Tryptophan	1.25	8.47E–42
5	12-hydroxyjasmonic acid glucoside	1.04	2.91E–35
6	Rosmarinyl glucoside	1.24	1.62E–06
7	Viscumneoside VI	1.42	9.35E–23
8	Eupatorin	1.76	1.38E–35
9	Luteolin	1.31	3.24E–40
10	Apigenin	1.23	4.50E–46
11	Luteolin 4'-methyl ether	1.23	1.97E–46
12	9(S)-HPODE	0.62	2.75E–04
13	LPE(C20:3)	1.28	1.07E–38
14	PI(C16:0)	0.90	3.46E–02
15	LPE(C18:2)	1.27	8.12E–29
16	Hydroxylinolenic acid	0.71	1.43E–02
17	LPE(C18:0)	1.21	5.52E–18
18	LPE(C18:2)	1.07	3.57E–22
19	Oleic acid	1.11	2.82E–02
20	Linoleic acid	1.29	3.02E–02
21	Linolenic acid	1.36	7.61E–03
22	Tocopherol	1.24	5.89E–18
23	Sitosterol	1.33	8.02E–12
24	3,5-octadien-2-one	1.13	2.41E–24

^aVariable importance in the projection (VIP) value were determined by PLS-DA

^b*p* value is retention indices calculated with *n*-Alkanes

according to either storage temperature or duration. The samples stored at 35 °C or 45 °C for 4 or 8 weeks were clearly distinct from the 1-week sample and the control, whereas no significant difference between the 4 and 8 weeks samples was observed (Fig. 3b). Considering only temperature, differences between the PSP samples stored for 4 or 8 weeks were significant. However, the differences among samples stored for 1 week at different temperatures were not statically significant (Fig. 3c).

Identification of major PSP metabolites

To identify specific metabolites including fatty acids that contributed to the observed differences in the PLS-DA score plots, the chromatographic peak intensities of all fatty acids and metabolites were analyzed. Twenty-four metabolites, including fatty acids, were identified (Table 1 and Supplementary Tables 1 and 2). Among them, maltose,

citric acid, isocitric acid, tryptophan, 12-hydroxyjasmonic acid-glucoside, rosmarinyl glucoside, viscumneoside VI, eupatorin, luteolin, apigenin, luteolin 4'-methyl ether, 9(S)-HPODE, LPE(C20:3), LPE(C18:2), LPE(C18:0), LPE(C16:0), PI(C16:0), hydroxylinolenic acid, oleic acid, linoleic acid, linolenic acid, tocopherol, sitosterol, and 3,5-octadien-2-one were significant ($p < 0.05$) based on storage duration and temperature. All identified fatty acids and most of the metabolites had VIP values > 0.8 with the exception of linoleic acid, linolenic acid, 9(S)-HPODE, and hydroxylinolenic acid. These results indicated that the fatty acids and metabolites were the main contributors to the observed differences.

Metabolomic pathways in PSP and the relative abundances of identified metabolites

A metabolomic pathway in PSP was constructed based on the metabolites that were identified, and primarily fatty acids and secondary metabolites were considered. The relative abundances of the metabolites and fatty acid compositions were compared according to temperature and storage duration (Fig. 4). The relative abundances of fatty acids in the samples stored for 1 and 4 weeks were lower than those in the control. The relative abundances of fatty acids in the samples stored for 8 weeks were similar to those in the control. Temperature did not appear to affect fatty acid abundances. However, the relative abundances of all phospholipids identified were significantly reduced with increasing temperature and storage duration. No LPEs were observed in samples stored at 45 °C for more than 4 weeks. In general, free fatty acids can be produced by degradation of lipid including phospholipid. Therefore, the degradation of LPEs in PSP might lead to the accumulation of free fatty acids (AV) (Fig. 1b). This would be in contrast to what has been observed in milled rice stored at 37 °C for 50 days with 70% RH (Lam and Proctor 2004). As was observed with the LPEs, the relative abundances of maltose, tryptophan, 12-hydroxyjasmonic acid, tocopherol, and sitosterol decreased with increasing storage duration and temperature. 12-hydroxyjasmonic acid was nearly absent in samples stored at 45 °C for more than 4 weeks. The relative abundances of maltose and tryptophan were two times lower than those in the control. Tocopherol and sitosterol, which are antioxidant phytochemicals (Yang et al. 2015), were reduced by between 20 and 58%. 12-Hydroxyjasmonic acid, known as a plant hormone related to plant stress response, is generally accumulated under stress and decreases within a couple hours (Miersch et al. 2008). Therefore, a decrease of 12-hydroxyjasmonic acid with increasing storage duration and temperature revealed that PSP was seriously damaged, leading to the change in the profiles of stress response-related compounds.

Fig. 4 Schematic representation of the PSP metabolic pathway over the duration of storage and the relative abundance of identified metabolites. The horizontal axis shows the normalized intensity. Different letters appearing within the same week indicated significant differences at $p < 0.05$

In contrast, the relative abundances of citric acid, 9(S)-HPODE, apigenin, luteolin, luteolin 4'-methyl ether, and 3,5-octadien-2-one were significantly higher in samples stored for more than 4 weeks at all temperatures. The highest concentrations were observed in samples stored for 4 or 8 weeks at 45 °C. Quantities in these samples were between 3- and 39-fold higher than those in samples stored for 1 week. Citric acid is associated with sour taste (Kang et al. 2011), while flavonoids (apigenin, luteolin, and luteolin 4'-methyl ether) confer various health benefits including antioxidant activity (Ali et al. 2017; Nijveldt et al. 2001). These compounds may be the products of enzymatic conversion of precursor metabolites, and they may prevent lipid oxidation. However, 3,5-octadien-2-one is one of the major lipid oxidation products from unsaturated fatty acids (Richards et al. 2005) and can be used as an indicator of lipid oxidation for PSP.

Unlike these flavonoids, rosmarinyl glucoside and viscumneoside VI, major secondary metabolites in perilla

seed, were lower in samples stored for more than 1 week. However, no further decrease was observed, and temperature did not appear to affect these compounds. Quantities of rosmarinic acid were similarly reduced, although the change was not statistically significant. In samples stored for 1 week at 25 °C and 35 °C, the quantity of eupatorine was elevated. The quantity of eupatorine decreased with longer storage times, but its concentration in samples stored at 45 °C was little changed. The quantity of total phenolic compounds did not appear to depend on either temperature or the duration of storage (Supplementary Fig. 1b).

Correlation between metabolites and quality factors

The correlations between metabolite concentrations in PSP, including fatty acids, and quality factors like lipid oxidation, color, and antioxidant activity, were calculated and visualized with a heat map (Fig. 5 and Supplementary

Fig. 5 Analysis of correlations between metabolites and quality parameters. The correlation matrix was analyzed and visualized with a heat map generated with the R corplot package. Positive correlations are shown in blue, and negative correlations are shown in red (color figure online)

Fig. 4). LPEs, phytosterols, tryptophan, 12-hydroxyjasmonic acid glucoside, and maltose correlated negatively with quality factors ($-0.96 < r < -0.53$) with the exception of the L^* value and antioxidant activity. Flavonoids (luteolin, apigenin, and luteolin 4'-methyl ester), citric acid, isocitric acid, 9(S)-HPODE, and 3,5-octadien-2-one showed a positive correlation ($0.41 < r < 0.91$) with quality factors. This indicated that these metabolites were the main contributors to changes in PSP quality, and that their concentrations depended on the duration of storage. In particular, lipid oxidation, as indicated by AV and POV, was promoted by the degradation of LPEs, resulting in accumulation of 3,5-octadien-2-one. Lipid oxidation might be mitigated by phytosterols and other antioxidants, phenolic compounds, and flavonoids during storage. However, the correlation between antioxidant activity measured by the DPPH· free radical scavenging method and antioxidant content was weak ($r < 0.33$).

Conclusion

To evaluate the effects of storage duration and temperature on the quality of PSP, changes in the metabolite profiles of PSP samples stored at 25 °C, 35 °C, and 45 °C was monitored over 8 weeks. Correlations between these changes and the quality parameters of lipid oxidation, color, and antioxidant activity were investigated. Metabolomic analysis revealed that all PSP samples stored at 25 °C and 35 °C and the sample stored for 1 week at 45 °C were clearly different from the control and samples stored at 45 °C for more than 4 weeks. Among the many metabolites contributing to these differences, LPEs, tocopherol, sitosterol, tryptophan, 12-hydroxyjasmonic acid glucoside, and maltose correlated negatively with the quality

parameters a^* , b^* , AV, and POV. In contrast, a positive correlation was found between these quality parameters and luteolin, apigenin, luteolin 4'-methyl ester, citric acid, isocitric acid, 9(S)-HPODE, and 3,5-octadien-2-one. PSP lipid oxidation was promoted by the hydrolysis of fatty acids and LPEs during storage, but the extent of oxidation was insufficient to cause rancidity within the storage period of 8 weeks. This was ascribed to the many antioxidant compounds in the samples, which included phytosterols, phenolic compounds, and flavonoids. Our results suggested that the quality of PSP samples stored at 25 °C, 35 °C, and 45 °C for 8 weeks could be considered acceptable, although the quantities of some antioxidants and lipids decreased during storage. Moreover, these metabolites including fatty acids could be useful for monitoring changes in PSP quality.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest to declare.

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