생활과학석사학위논문

Flavonoids in Common and Tartary Buckwheat Hull Extracts and Antioxidant Activity of the Extracts against Lipids in Mayonnaise

단메밀과 쓴메밀 껍질 추출물에 함유된 플라보노이드 조성 및 메밀 껍질 추출물에 의한 마요네즈의 지질 산화 안정성

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ABSTRACT

Flavonoids in Common and Tartary Buckwheat Hull Extracts and Antioxidant Activity of the Extracts against Lipids in Mayonnaise

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Buckwheat hulls, generally discarded as waste, have been known to possess various flavonoids and high antioxidant activities. However, studies on the flavonoid of common and tartary buckwheat hulls and their application are still limited. The objective of this study was to determine the effect of extracting solvents (water, ethanol (20%, 50%, 80%, and 100%), methanol, and acetone) on flavonoid composition and content and to analyze antioxidant activities of common and tartary buckwheat hull extracts. Antioxidative effect of common and tartary buckwheat hull extracts on lipids in mayonnaise was also investigated.

Vitexin, isovitexin, isoorientin, orientin, rutin, isoquercetin, and quercetin were identified in the common buckwheat hull extracts, while rutin,

quercetin, isoorientin, and isoquercetin were in the tartary buckwheat hull extracts. Vitexin was more extracted from common buckwheat hulls when using methanol than the other solvents. Rutin, the major flavonoid in the tartary buckwheat hull extracts, was much more detected in the 80% ethanol and methanol extracts than in the others. Total phenolic content and antioxidant activities were higher in the aqueous ethanol extracts from both of the hulls.

Common and tartary buckwheat hull extracts using 50% ethanol were applied in mayonnaise at 0.02 and 0.08% (w/w). Peroxide value, 2-thiobarbituric acid value, *p*-anisidine value, and total oxidation value of the mayonnaises stored at 35 °C for 31 days were determined. Addition of the common and tartary buckwheat hull extracts retarded lipid oxidation in mayonnaise. In conclusion, common and tartary buckwheat hull extracts possess high antioxidant activity with various flavonoids and they may be used as efficient antioxidants for mayonnaise.

Keywords: Buckwheat hull, Flavonoid, Antioxidant activity, Solvent extraction, Mayonnaise

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INTRODUCTION

Buckwheat, a pseudocereal which belongs to *Polygonaceae*, is consumed worldwide. Common (*Fagopyrum esculentum*) and tartary buckwheats (*F. tataricum*) are the most commonly cultivated species around the world (Zhang et al., 2012). Buckwheat has been known to have a large amount of rutin (quercetin 3-rutinoside) with other flavonoids (Li et al., 2010). Flavonoids have received considerable attention because of their beneficial effects on health such as antioxidant, antitumor, antihypertensive, and anti-inflammatory activities (Kumar and Pandey, 2013).

Buckwheat whole grains are generally dehulled to produce groats which are used for human consumption either in groats themselves or flour (Zielinski et al., 2009). A substantial quantity of hulls resulted from dehulling process of buckwheat is discarded as waste, which could be considered as a source of flavonoids (Lee et al., 2016).

Previous studies have reported that buckwheat hulls contain high levels of phenolics and flavonoids and their levels are even higher than dehulled buckwheat groats (Dziadek et al., 2016; Holasova et al., 2002; Lu et al., 2013; Sedej et al., 2012). Moreover, buckwheat hulls have higher antioxidant activity than buckwheat groats, regardless of cultivars (Dziadek et al., 2016). Although buckwheat hulls are rich in flavonoids, studies on buckwheat hulls are still limited.

Solvent extraction has been widely used to extract flavonoids from plants (Liu et al., 2007). The most commonly used solvents for extracting flavonoids from plant foods are water, aqueous ethanol, methanol, and acetone (Xu and Chang, 2007). Composition and content of flavonoids in the extracts generally depend on the type of solvent, and thus their biological activity may vary. Therefore, it is important to select an appropriate solvent for extracting flavonoids with a high efficiency of extraction (Kajdzanoska et al., 2011). However, there have been no published data investigating influence of solvent on composition and content of flavonoids in buckwheat hull extracts as well as their antioxidant activities.

Mayonnaise is an oil-in-water (O/W) emulsion, in which 70-80% of oil (the dispersed phase) is dispersed in water (the continuous phase) with egg yolk as an emulsifier at the interface (Li et al., 2014). In O/W emulsion, lipid oxidation may take place at the interface between oil and water, where pro-oxidants in the continuous phase are able to contact with the hydroperoxides at the droplet surface (McClements et al., 2000). Ultimately, lipid oxidation causes undesirable off-flavors and decreases shelf life of products (Gorji et al., 2016). In order to retard lipid oxidation in foods, synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and ethylene diamine tetraacetic acid (EDTA) have been commonly used in mayonnaise (Gorgi et al., 2016; Kwon et al., 2015).

However, consumers have negative perception towards these synthetic antioxidants (Li et al., 2014). Therefore, there has been a growing interest in natural antioxidants from plant materials rich in phenolic compounds. Several studies on applying natural antioxidants to mayonnaise such as rapeseed cake extract (Kim and Lee, 2017), purple corn extract (Li et al., 2014), grape seed extract (Altunkaya et al., 2013), and black glutinous rice extract (Tananuwong et al., 2010) have been conducted. Although buckwheat hulls, which are known to contain high levels of phenolic compounds, including flavonoids, are expected to have antioxidative effect on lipids in mayonnaise, it has not yet been studied.

Thus, the objective of this study was to determine the effect of various extracting solvents (water, ethanol (20%, 50%, 80%, and 100%), methanol, and acetone) on flavonoid composition and content and to analyze antioxidant activities of common and tartary buckwheat hull extracts. Antioxidative effect of common and tartary buckwheat hull extracts on lipids in mayonnaise was also investigated.

MATERIALS AND METHODS

1. Materials and chemicals

Common and tartary buckwheat hulls collected from Bongpyeong (Korea) in September, 2017 were obtained. The buckwheat hulls were dried in a freeze dryer (FD8512, IlShinBioBase Co., Yangju, Korea) for 2 days and stored at -20 °C until analyzed.

Soybean oil, egg yolk, vinegar, salt, and sugar were purchased from local markets in Seoul, Korea. Ethanol, methanol, acetone, sodium bicarbonate anhydrous, formic acid, acetic acid, chloroform, potassium iodide, isooctane, and 1-butanol were purchased from Samchun Pure Chemicals (Pyeongtaek, Korea). Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, gallic acid, rutin, isoquercetin, quercetin, butylated hydroxytoluene (BHT), *p*-anisidine, and 2-thiobarbituric acid were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Isoorientin, orientin, isovitexin, and vitexin were purchased from Chem Faces (Wuhan, China). Acetonitrile was purchased from JT Baker (Phillipsburg, NJ, USA). Starch was from Wako Pure Chemical Co. (Osaka, Japan). Sodium thiosulfate was purchased from Junsei Chemical Co. (Tokyo, Japan). All chemicals were of analytical reagent grade.

2. Extraction of buckwheat hulls

Dried buckwheat hulls were pulverized using a blender (Hanil Co., Bucheon, Korea) to get 18 mesh (1 mm) size powder. The ground buckwheat hull powder (10 g) was refluxed with 400 mL water, ethanol (20, 50, 80, and 100%), methanol, or acetone for 2 h in a water bath (Daihan Scientific Co., Seoul, Korea). The extract was filtered through a Whatman No. 4 filter paper (Whatman International Ltd., Maidstone, England) and the filtrate was concentrated using a rotary evaporator (A-10005, Eyela Co., Tokyo, Japan) at 50 °C. The concentrated extract was freeze-dried and then stored at -20 °C until further analysis. Yield of the buckwheat hull extract was calculated as follows:

Yield (%) = $(W_1/W_0) \times 100$,

where W_0 is weight of buckwheat hull (g, dry basis) and W_1 is weight of freeze-dried extract (g).

Dried buckwheat hull extract was reconstituted in the corresponding solvent for the following assays.

3. Determination of total phenolic content (TPC)

TPC of the extract was determined by the method of Singleton et al. (1999) with a slight modification. The buckwheat hull extract (40 μL) was mixed with 3.16 μL water and 200 μL Folin-Ciocalteu reagent. After 3 min, the mixture was reacted with 600 μL 20% (w/v) sodium bicarbonate

solution and incubated for 30 min at 40 °C. Absorbance was measured at 765 nm. TPC was expressed as gallic acid equivalent (GAE).

4. HPLC-ESI-MS and HPLC-UV analyses of flavonoids in buckwheat hull extracts

Flavonoids were identified by HPLC-MS using an Ultimate 3000 RS HPLC system coupled with an LTQ XL (Thermo Fisher Scientific, Waltham, MA, USA). The buckwheat hull extract (2 mg/mL) was filtered with 0.2 μm nylon syringe filter. Electrospray ionization (ESI) negative ion mode ([M-H]-) was applied. A U-VDSpher PUR C18-E column (100 × 2 mm, 1.8 μm, VDS Optilab, Berilin, Germany) was used for separation. Mobile phases were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Flow rate was 0.3 mL/min. Injection volume was 5 μL with a gradient as follows: 0-1min, 5% B; 1-15 min, 5-25% B; 15-21 min, 25-60% B; 21-22 min, 60-100% B; 22-23 min, 100% B; 23-24 min, 100-5% B; and 24-30 min, 5% B. Data were acquired in scan mode using a m/z range of 100 to 1000. Mass parameters were set as follows: capillary temperature, 300 °C; source voltage, 2.7 kV; sheath gas flow, 42; and software, Xcalibur 4.0 (Thermo Fisher Scientific, Waltham, MA, USA).

Quantification of flavonoids in the extracts was carried out using reversed-phase HPLC (Ultimate 3000; Thermo Scientific Dionex, Waltham, MA, USA) equipped with an XBridge C18 column (4.6 × 250 mm, 5 μm,

Waters, USA). Mobile phases were 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Flow rate was 0.8 mL/min. Injection volume was 20 μL with a gradient as follows: 0-7min, 1-5% B; 7-45 min, 5-50% B; 45-52 min, 50-95% B; 52-56 min, 95-1% B; and 56-70 min, 1% B. Column oven temperature was at 50 °C. Detection wavelength was set at 350 nm. Five-level calibration curve was generated by analysis of known concentrations (0.25 – 50 μg/mL) of each standard.

5. Antioxidant activities of buckwheat hull extracts

DPPH free radical scavenging activity was measured according to a modified method from Brand-Williams et al. (1995). 125 μL of the extract (50 μg/mL) was mixed with 125 μL 0.2 mM DPPH dissolved in methanol. After 30 min at room temperature in the dark, absorbance was measured at 517 nm. ABTS free radical scavenging activity was determined by the method of Re et al. (1999). To make ABTS solution, 7 mM ABTS solution and 2.45 mM potassium persulfate solution were mixed at a ratio of 1:1 and kept overnight at room temperature. ABTS solution was diluted with water to an absorbance of less than 0.70 at 734 nm before use. Ten μL of the extract (500 μg/mL) was mixed with 1 mL of the diluted ABTS solution. Absorbance was measured at 734 nm. DPPH or ABTS free radical scavenging activity (%) was calculated as follows:

DPPH or ABTS free radical scavenging activity (%) = (1 – sample

absorbance/control absorbance) × 100.

6. Preparation and storage of mayonnaise

Mayonnaise was prepared with soybean oil, egg yolk, vinegar, salt, sugar, and water as in Table 1. Based on the TPC and antioxidant activities of the crude extracts, 50% ethanol extract was chosen to be applied in mayonnaise. Common and tartary buckwheat hull extracts were added to the mayonnaise at 0.02% (w/w) and 0.08%. Control sample was prepared without buckwheat hull extracts or BHT. BHT was added at 0.02% as a positive control.

Mayonnaise was prepared as follows: all the ingredients except oil were mixed using a hand blender (Wiz Co., Daegu, Korea) for 20 sec. The oil was slowly added to the mixture blending for 2 min. The mayonnaise (30 g) was transferred into a 50 mL cylindrical polystyrene tube, sealed with a screw cap, and stored at 35 °C for 31 days. The samples were prepared in triplicate (3 batches per treatment) and each sample was used only once for the measurement.

Table 1. Formulation of mayonnaise

Ingredient	% (w/w)					
	Control	CE8	CE2	TE8	TE2	BHT
Soybean oil	75	75	75	75	75	75
Egg yolk	9	9	9	9	9	9
Vinegar	6.5	6.5	6.5	6.5	6.5	6.5
Antioxidant	-	0.08	0.02	0.08	0.02	0.02
Salt	1.5	1.5	1.5	1.5	1.5	1.5
Sugar	2	2	2	2	2	2
Water	6	5.92	5.98	5.92	5.98	5.98

CE8: added with 0.08% (w/w) common buckwheat hull extract powder; CE2: added with 0.02% common buckwheat hull extract powder; TE8: added with 0.08% tartary buckwheat hull extract powder; TE2: added with 0.02% tartary buckwheat hull extract powder; and BHT: added with 0.02% butylated hydroxytoluene

7. Lipid extraction from mayonnaise

Lipid was extracted by method of Lagunes-Galvez et al. (2002) with a slight modification. Mayonnaise was frozen at -74 °C for 24 h and thawed for 2 h at room temperature to break the emulsion. The thawed mayonnaise was centrifuged (2236R, Gyrozen Co., Daejeon, Korea) at 18,000 × *g* at 15 °C for 10 min. The lipid phase was used immediately after the separation for measuring oxidative stability of lipids in mayonnaise.

8. Oxidative stability of lipids in mayonnaise

Progression of lipid oxidation in mayonnaise was monitored by determining peroxide value (PV), *p*-anisidine value (*p*-AV), 2-thiobarbituric acid (TBA) value, and total oxidation (Totox) value. PV, *p*-AV, and TBA value were determined by AOCS Official Method (2009) Cd 9-53, Cd 19-90, and Cd 19-90, respectively. Totox value was calculated by 2PV + *p*-AV (Sherwin 1978).

9. Statistical analysis

Data were expressed as means ± standard deviations. Independent t-test and one-way analysis of variance (ANOVA) with Duncan's multiple range test ($p < 0.05$) were performed with SPSS 23.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

1. Yields of buckwheat hull extracts

Extraction yields of the common and tartary buckwheat hulls ranged from 0.6 to 5.8% (w/w, dry basis) and from 0.4 to 4.4%, respectively (Table 2). Water extracted the most from both of common and tartary buckwheat hulls, while acetone did the least.

2. TPC in buckwheat hull extracts

TPC in the common and tartary buckwheat hull extracts were affected by the solvents as shown in Fig. 1. The highest TPC was observed in the 20% ethanol extracts followed by the 50% ethanol and 80% ethanol extracts. Yilmaz and Toledo (2006) reported that an organic solvent containing water was better than the solvent alone when extracting phenolic compounds from muscadine seeds. Lapornik et al. (2005) also demonstrated that more phenolic compounds are extracted by 70% ethanol and 70% methanol than by water. These results imply that an organic solvent containing water more efficiently extracts phenolic compounds than the individual organic solvent.

Table 2. Yields of buckwheat hull extracts

Solvent	Yield (%, w/w)	
	Common	Tartary
Water	5.82±0.44[a]	4.39±2.48[a]
20% Ethanol	4.11±0.36[b]	3.38±0.07[a]
50% Ethanol[*]	4.20±0.22[b]	3.40±0.07[a]
80% Ethanol	2.75±0.11[c]	2.73±0.03[ab]
Ethanol[*]	2.01±0.22[d]	1.04±0.06[bc]
Methanol[**]	2.44±0.30[cd]	1.47±0.10[bc]
Acetone	0.59±0.23[e]	0.41±0.02[c]

Values are means ± standard deviations (n=3).

[a,b,c,d,e]Different superscripts indicate significant differences within the same columns ($p < 0.05$; one-way ANOVA and Duncan's multiple range test).

[*,**]Significant difference between common and tartary buckwheat hull extracts ($p < 0.05$ or 0.01; independent t-test).

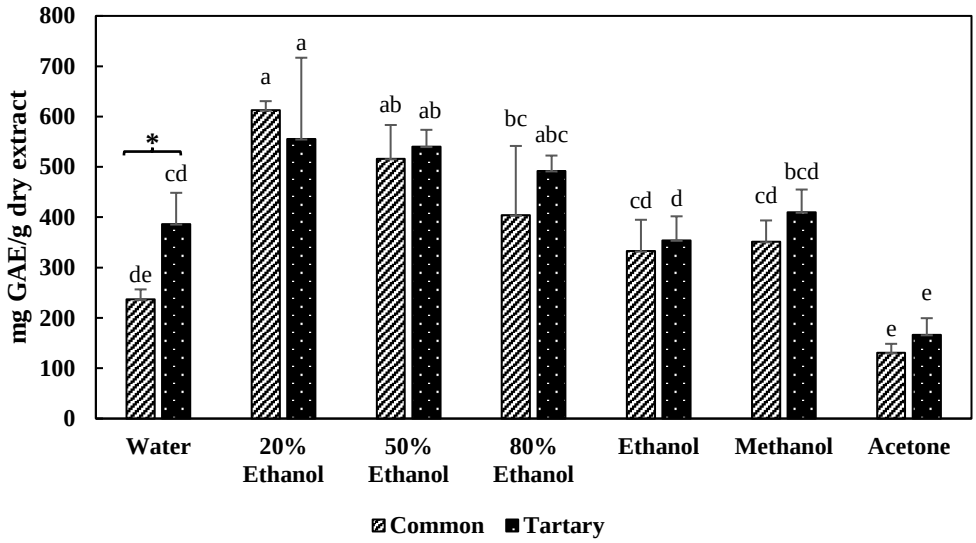

Figure 1. Total phenolic content in buckwheat hull extracts prepared by various solvents

GAE: gallic acid equivalent.

Values are means ± standard deviations (n=3).

[a,b,c,d,e]Different letters indicate significant differences within the same buckwheat hull extracts ($p < 0.05$; one-way ANOVA and Duncan's multiple range test).

*Significant difference between common and tartary buckwheat hull extracts ($p < 0.05$; independent t-test).

3. Identification of flavonoids in buckwheat hull extracts

HPLC-ESI-MS was used to identify major flavonoids present in the common and tartary buckwheat hull extracts. MS spectra of common and tartary buckwheat hull extracts are shown in Fig. 2 and 3, respectively. Flavonoids were identified matching the peaks observed by mass spectra. Isoorientin, orientin, rutin, isovitexin, vitexin, isoquercetin, and quercetin were identified in the common buckwheat hull extracts, while isoorientin, rutin, isoquercetin, and quercetin were in the tartary buckwheat hull extracts. Peak 1 and 2 produced m/z 447 of $[M-H]^-$ on MS, which was identified as isoorientin and orientin, respectively. Peak 3 was determined as rutin at m/z 609 $[M-H]^-$ and peak 4 and 5 were as isovitexin and vitexin, respectively, at m/z 431 $[M-H]^-$. Peak 6 with m/z 463 $[M-H]^-$ was determined as isoquercetin. Peak 7 which had m/z 301 $[M-H]^-$ was identified as quercetin. Lee et al. (2016) identified eight major flavonoids in common and tartary buckwheat hulls, including rutin, quercetin, vitexin, isovitexin, orientin, isoorientin, catechin, and eqicatechin gallate. However, catechin and epicatechin gallate were not detected in the present study, but isoquercetin was.

4. Composition of flavonoids in buckwheat hull extracts

Individual flavonoids in the common and tartary buckwheat hull extracts were quantified using their corresponding standards (Fig. 4). The common

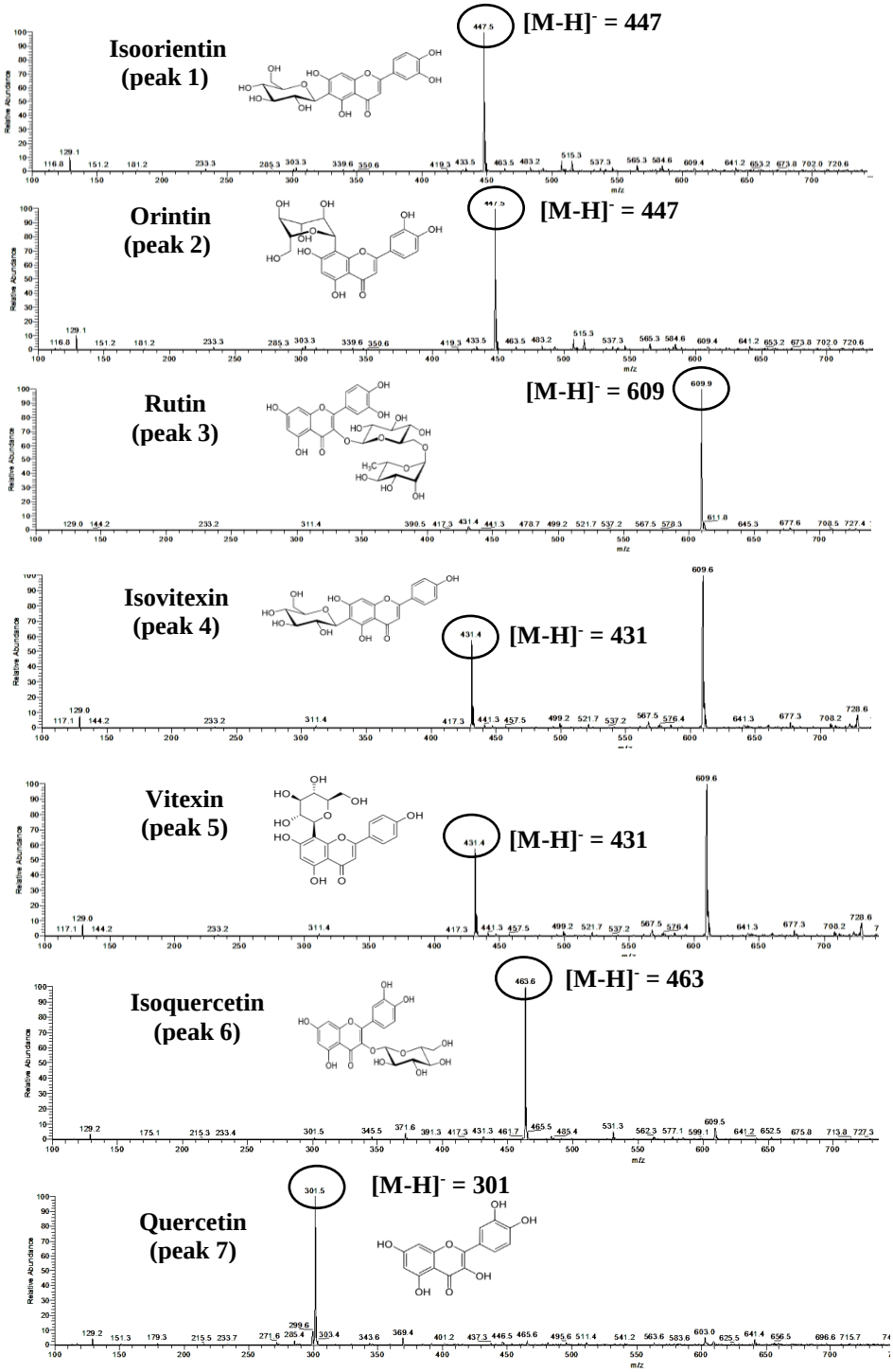

Figure 2. HPLC-ESI-MS spectra of flavonoids in common buckwheat hull extracts with negative ion mode.

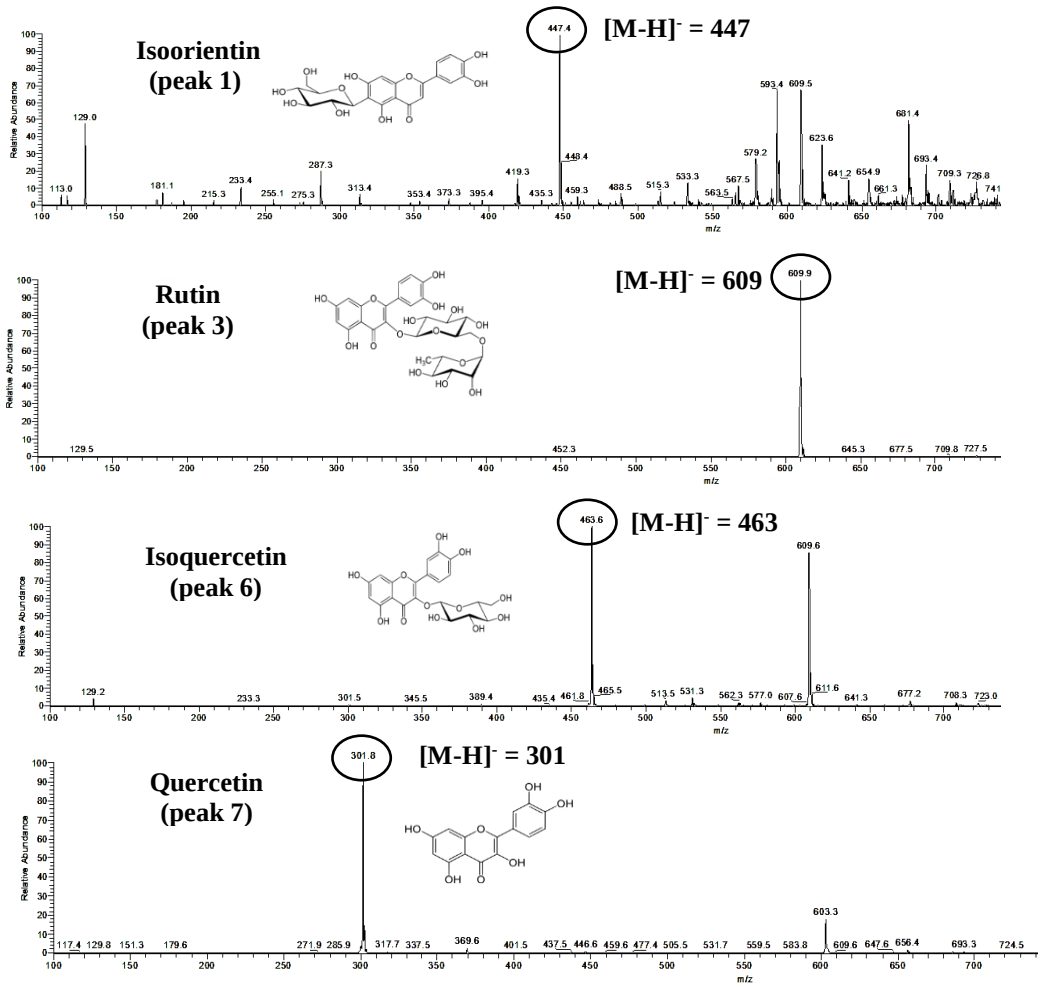

Figure 3. HPLC-ESI-MS spectra of flavonoids in tartary buckwheat hull extracts with negative ion mode.

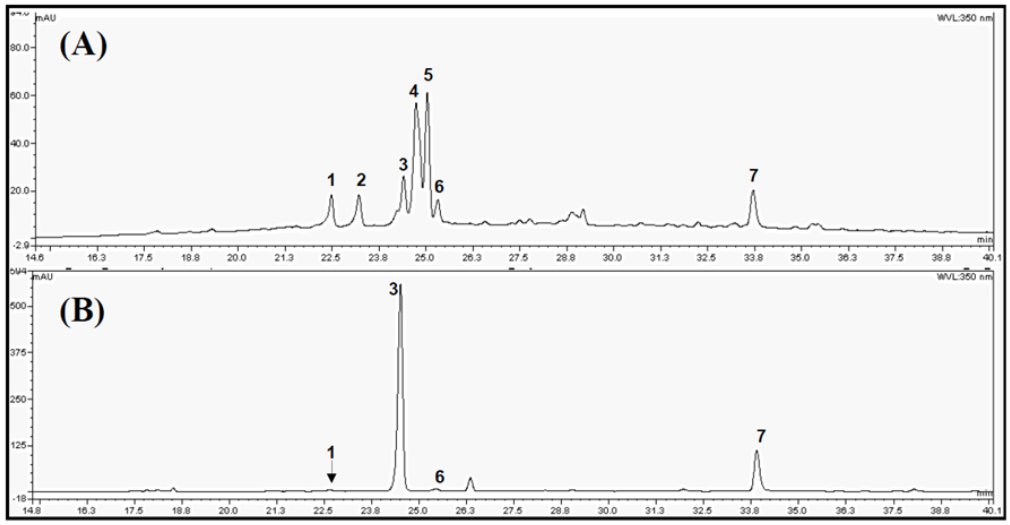

Figure 4. HPLC chromatograms analyzing flavonoids in (A) common and (B) tartary buckwheat hull extracts (λ=350 nm). The peaks represent 1, isoorientin; 2, orientin; 3, rutin; 4, isovitexin; 5, vitexin; 6, isoquercetin; and 7, quercetin.

buckwheat hull extracts contained more diverse flavonoids than the tartary buckwheat hull extracts (Table 3). Major flavonoids in the common buckwheat hull extracts were vitexin and isovitexin. Rutin has been known to be the representative flavonoid in buckwheat. In this study, however, vitexin and isovitexin were detected in larger amounts than rutin in the common buckwheat hull extracts. Sedej et al. (2012) reported the most abundant flavonoid in whole grains, hulls, and groats is rutin. Similarly, Lee et al. (2016) suggested that rutin is the major flavonoid in both common and tartary buckwheat hulls. However, Zhang et al. (2017) reported that the major flavonoids vary depending on the cultivars. Vitexin and isovitexin, the major flavonoids in the common buckwheat hull extracts, have been considered to have antioxidant (Kim et al., 2005), anti-inflammatory (Borghi et al., 2013), anti-thyroid (Gaitan et al., 1995), and anti-tumor (Choi et al., 2006) activities. Vitexin, isovitexin, and rutin were highly extracted when using methanol (12.24, 7.36, and 5.37 mg/g, respectively).

Isoorientin and orientin have been reported to exist only in buckwheat hulls rather than in groats (Lee et al., 2016). In this study, isoorientin and orientin were both detected in common buckwheat hulls and highly extracted when using 50% ethanol (1.35 and 2.57 mg/g, respectively). Vrinda and Devi (2001) reported that orientin provides protection against DNA and bone marrow damage. In addition, ABTS radical scavenging activity of orientin was found to be much higher than those of rutin,

Table 3. Composition of major flavonoids in buckwheat hull extracts

	Solvent	Content (mg/g dry extract)							
		Isoorientin	Orientin	Rutin	Isovitexin	Vitexin	Isoquercetin	Quercetin	Total
Common	Water	0.41±0.22^{c}	1.55±0.36^{c}	1.23±0.30^{b}	1.35±0.49^{d}	6.70±2.40^{bc}	1.38±0.55^{ab}	0.21±0.05^{e}	12.8±4.4^{c}
	20% Ethanol	0.90±0.11^{b}	2.21±0.04^{b}	0.89±0.09^{bc}	4.13±0.23^{c}	5.44±0.22^{c}	1.28±0.17^{ab}	0.56±0.23^{d}	15.4±0.9^{c}
	50% Ethanol	1.35±0.15^{a}	2.57±0.17^{a}	1.30±0.13^{b}	5.97±0.72^{b}	8.25±0.69^{b}	1.88±0.19^{a}	1.54±0.17^{b}	22.9±2.0^{b}
	80% Ethanol	0.82±0.13^{b}	1.69±0.05^{c}	5.04±0.30^{a}	6.45±0.35^{b}	6.89±0.47^{bc}	1.25±0.40^{b}	2.70±0.16^{a}	24.8±1.2^{ab}
	Ethanol	0.22±0.06^{d}	0.63±0.03^{e}	4.73±0.86^{a}	3.41±0.66^{c}	5.25±2.06^{c}	0.89±0.36^{c}	1.21±0.25^{c}	16.3±4.1^{c}
	Methanol	0.50±0.12^{c}	1.16±0.02^{d}	5.37±0.39^{a}	7.36±0.49^{a}	12.24±1.06^{a}	1.37±0.25^{ab}	1.29±0.19^{bc}	29.3±2.0^{a}
	Acetone	ND	0.33±0.02^{f}	0.42±0.08^{c}	0.37±0.03^{e}	0.87±0.09^{d}	0.27±0.02^{d}	0.26±0.03^{de}	2.5±0.1^{d}
Tartary	Water	0.44±0.05^{b}	ND	43.12±0.14^{c}	ND	ND	2.14±0.26^{b}	0.83±0.05^{d}	46.5±0.5^{d}
	20% Ethanol	0.51±0.07^{b}	ND	53.41±0.75^{b}	ND	ND	3.16±0.05^{a}	3.02±0.32^{d}	60.1±0.5^{c}
	50% Ethanol	0.96±0.20^{a}	ND	60.03±3.29^{ab}	ND	ND	3.76±0.33^{a}	15.00±1.03^{c}	79.7±4.4^{b}
	80% Ethanol	0.32±0.15^{b}	ND	68.49±9.45^{a}	ND	ND	1.98±0.60^{b}	20.27±2.23^{b}	91.1±11.0^{ab}
	Ethanol	ND	ND	36.90±9.10^{c}	ND	ND	1.96±0.77^{b}	27.90±3.05^{a}	66.8±11.5^{c}
	Methanol	ND	ND	65.95±5.33^{a}	ND	ND	3.36±0.83^{a}	28.23±2.07^{a}	97.5±6.9^{a}
	Acetone	ND	ND	7.80±2.19^{d}	ND	ND	1.51±0.16^{b}	11.73±2.84^{c}	21.0±5.1^{e}

Values are means ± standard deviations (n=3). ^{a,b,c,d,e,f}Different superscripts indicate significant differences within the same columns and the same buckwheat hull extracts ($p < 0.05$; one-way ANOVA and Duncan's multiple range test). ND, not detected.

quercetin, vitexin, isovitexin, and catechin (Lee et al., 2016). Yuan et al. (2012) reported that isoorientin possesses therapeutic and chemopreventive effects against liver cancer.

It has been reported that buckwheat hulls contain various flavonoids, while buckwheat groats only contain rutin and isovitexin (Dietrych-Szostak and Oleszek, 1999; Krahl et al., 2008). In this study, various functional flavonoids were also detected in the common buckwheat hull extracts, suggesting that the hulls could be a source with various physiological functions.

Unlike in the common buckwheat hull extracts, only isoorientin, rutin, isoquercetin, and quercetin were detected in the tartary buckwheat hull extracts (Table 3). In the tartary buckwheat hull extracts, rutin was the major flavonoid in agreement with the results of Guo et al. (2012) and Lee et al. (2016). Rutin detected in the tartary buckwheat hull extracts (7.80–68.5 mg/g) was at least 10 times higher than that in the common buckwheat hull extracts (0.42–5.37 mg/g). Lee et al. (2016) reported composition and content of major flavonoids in both groats and hulls of common and tartary buckwheat, where lower levels of rutin in common and tartary buckwheat hulls (0.19 and 1.99 mg/g, respectively) were detected than in this study.

Quercetin, the second largest flavonoid in the tartary buckwheat hull extracts, was more detected in the methanol and ethanol extracts (28.2 and 27.9 mg/g, respectively), whereas it was least detected in the water extract

(0.83 mg/g). This result is similar to the findings of Vasantha et al. (2011), who observed that quercetin was not detected in water extract, but only in methanol and acetone extracts. Cacace and Mazza (2003) also reported that quercetin has low solubility in water. As with the results of rutin, quercetin in the tartary buckwheat hull extracts was much more than in the common buckwheat hull extracts except for the water extract.

Isoquercetin, a glycosylated flavonoid derived from quercetin, was detected in both of the common and tartary buckwheat hull extracts. Unlike quercetin, isoquercetin was highly detected in the 50% ethanol extracts (1.88 and 3.76 mg/g for the common and tartary buckwheat hull extracts, respectively). It might be because a glucoside, which constitutes isoquercetin, is considered to be a polar molecule that is better extracted in aqueous ethanol than in ethanol alone (Khiari et al., 2009). Zhang et al. (2011) reported that isoquercetin has a regulative role in blood glucose and lipid levels. Moreover, Morand et al. (2000) demonstrated that isoquercetin is better absorbed than quercetin in rats.

Isoorientin in tartary buckwheat hulls was extracted only when using water or aqueous ethanol. Among the solvents used, 50% ethanol was the most effective in extracting isoorientin from both of common and tartary buckwheat hulls.

Among the solvents used in this study, methanol (29.3 and 97.5 mg/g in the common and tartary buckwheat hull extracts, respectively) extracted the

most amount of flavonoids followed by 80% ethanol (24.8 and 91.1 mg/g in the common and tartary buckwheat hull extracts, respectively) and 50% ethanol (22.9 and 79.7 mg/g in the common and tartary buckwheat hull extracts, respectively). Acetone was the poorest in extracting flavonoids (2.5 and 21.0 mg/g in the common and tartary buckwheat hull extracts, respectively). These results suggest that extracting solvents affect content and composition of flavonoids in buckwheat hull extracts.

5. Antioxidant activities of buckwheat hull extracts

Extracting solvents affected antioxidant activities of the buckwheat hull extracts (Table 4). Both of the DPPH and ABTS radical scavenging activities were higher in the aqueous ethanol extracts. The common and tartary buckwheat hull extracts prepared by 20% and 50% ethanol exhibited significantly ($p < 0.05$) higher DPPH and ABTS radical scavenging activities than by the other solvents. Meanwhile, the acetone extracts showed significantly ($p < 0.05$) lower DPPH and ABTS radical scavenging activities. In this study, the extracts with more TPC showed higher antioxidant activities. Holasova et al. (2002) reported that there were statistically significant relationships between total phenolics and antioxidant activity. Velioglu et al. (1998) also demonstrated the correlation between

Table 4. Antioxidant activities of buckwheat hull extracts

Solvent	DPPH radical scavenging activity (%)		ABTS radical scavenging activity (%)	
	Common	Tartary	Common	Tartary
Water	67.7±2.81[b]	72.07±5.12[b]	75.9±5.4[b]	77.6±1.88[c]
20% ethanol	86.5±2.88[a]	81.8±2.73[a]	95.4±0.6[a]	90.3±2.06[a]
50% ethanol	85.8±1.55[a]	82.0±2.14[a]	95.4±0.9[a]	85.9±1.99[b]
80% ethanol	71.3±0.99[b]	72.2±4.31[b]	92.2±1.0[a]	83.8±3.04[b]
Ethanol	39.9±2.95[d]	38.7±3.29[d]	74.0±3.1[b]	65.1±0.69[d]
Methanol	57.1±5.98[c]	50.3±4.34[c]	74.1±2.7[b]	67.1±1.62[d]
Acetone	23.4±2.01[e]	8.92±1.88[e]	21.0±4.1[c]	32.6±2.65[e]

Values are means ± standard deviations (n=3).

[a,b,c,d,e]Different superscripts indicate significant differences within the same columns ($p < 0.05$; one-way ANOVA and Duncan's multiple range test).

TPC and antioxidant activity in various plant materials. However, sum of the major flavonoids determined in this study was significantly ($p < 0.05$) higher in the methanol and 80% ethanol extracts than in the 20% and 50% ethanol extracts. A similar result was also reported by Vuong et al. (2013), who observed flavonoids were more extracted in organic solvents than water, while polyphenol yield and antioxidant properties were significantly higher in water extract. This inconsistency might result from the fact that other phenolic compounds, which have not been identified, may also contribute to the antioxidant activity (Lou et al., 2014).

Based on the results, the 50% ethanol extract, possessing high TPC and various flavonoids, as well as high antioxidant activities, was selected to be used for the study on the oxidative stability of lipids in mayonnaise.

6. Oxidative stability of lipids in mayonnaise

Oxidative stability of lipids in mayonnaise was determined under accelerated oxidation conditions at 35 ˚C during storage for 31 days (Fig. 5). PV of lipids in mayonnaise added with buckwheat hull extracts were considerably lower than those of the control and BHT-added mayonnaise during storage. PV of lipids in mayonnaises were initially 0.5-0.6 meq/kg and gradually increased. However, by the 26th day, the control

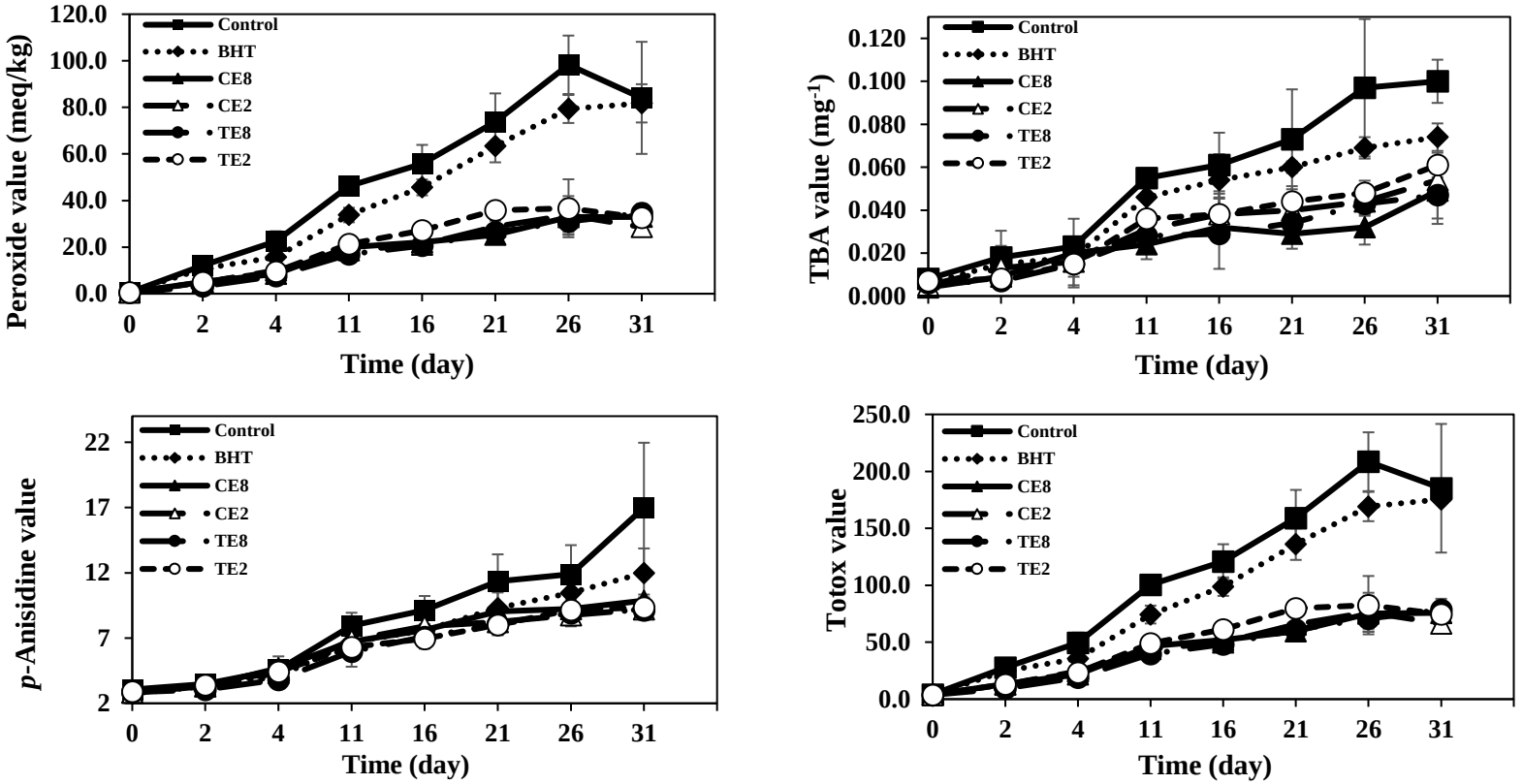

Figure 5. Oxidation levels in the lipid phase of mayonnaise during storage at 35 °C for 31 days.

Values are means ± standard deviations (n=3).

CE8: added with 0.08% (w/w) common buckwheat hull extract powder; CE2: added with 0.02% common buckwheat hull extract

powder; TE8: added with 0.08% tartary buckwheat hull extract powder; TE2: added with 0.02% tartary buckwheat hull extract powder; BHT: added with 0.02% butylated hydroxytoluene; TBA: 2-thiobarbituric acid; and Totox: total oxidation

mayonnaise reached the highest PV of 98.3 meq/kg and decreased to 84.1 meq/kg at the 31st day. This could be due to degradation of peroxides into secondary oxidative product (Gorji et al., 2016; Kim and Lee, 2017). Although PV of the mayonnaise added with BHT was lower than that of the control, those added with buckwheat hull extracts were even lower, suggesting that the buckwheat hull extracts might retard oxidation in early stages. Yi et al. (2017) reported that rutin and quercetin have ability to increase oxidative stability of lipids in an O/W emulsion. It is, therefore, suggested that flavonoids such as rutin and quercetin in buckwheat hull extracts may contribute to retard the lipid oxidation in mayonnaise.

TBA values of all the mayonnaise samples continuously increased during storage, implying that primary products are continuously decomposed into secondary products. As with the result of PV, TBA values of lipids in the mayonnaises added with buckwheat hull extracts were lower than those of the control and BHT-added mayonnaise. The lowest TBA value (0.047) was observed in the mayonnaise added with the tartary buckwheat hull extracts (0.08%) and the highest TBA value (0.1) was in the control on the 31st day.

p-AV of lipids in all the prepared mayonnaise samples gradually increased with storage time. *p*-AV of the control increased rapidly after the 26th day, when the highest PV was observed. This result could be due to generation of secondary oxidation products resulted from degradation of peroxides (Kim and Lee, 2017) as mentioned earlier. However, the

mayonnaises added with BHT and buckwheat hull extracts showed lower *p*-AV than the control between the 11th and 31st days during the storage, implying that BHT and buckwheat hull extracts might delay the development of secondary oxidation products.

Totox values of all the samples increased during storage except for the control, which started to decrease on the 26th day as with the result of PV. Totox value of the mayonnaise added with BHT was much higher than those of mayonnaises added with the buckwheat hull extracts, indicating that the addition of 0.02 and 0.08% common and tartary buckwheat hull extracts in mayonnaise could more effectively retard lipid oxidation than that of BHT. Similarly, Li et al. (2014) reported that purple corn husk extract-added mayonnaise demonstrated lower PV, p-AV, Totox value than BHT and EDTA-added mayonnaise.

Both of the common and tartary buckwheat hull extracts retarded the lipid oxidation in mayonnaise. Although, the composition and content of major flavonoids were clearly different between the common and tartary buckwheat hull extracts, PV, *p*-AV, TBA value, and Totox value were not significantly ($p > 0.05$) different among the mayonnaises added with buckwheat hull extracts. This is consistent with the results of TPC and antioxidant activity, which also showed no significant ($p > 0.05$) differences between the common and tartary buckwheat hull extracts using 50% ethanol. These results suggest that not only major flavonoids but also other

phenolic compounds in buckwheat hull extracts might contribute to antioxidative effect on lipid oxidation in mayonnaise. Bholah et al. (2015) also observed that *Moringa oleifera* leaf extracts, which are lower in flavonoids and higher in TPC than pod extracts, showed better antioxidant effect against lipids in mayonnaise than pod extracts.

CONCLUSION

Types of extracting solvents considerably affected yield, TPC, and flavonoid composition and content of common and tartary buckwheat hull extracts, as well as their antioxidant activities. The methanol and 80% ethanol extracts had greater quantities of flavonoids, while the 20% and 50% ethanol extracts had more TPC and higher antioxidant activities. Addition of common and tartary buckwheat hull extracts (0.02 and 0.08%) efficiently increased oxidative stability of lipids in mayonnaise when PV, *p*-AV, TBA, and Totox value were determined. Therefore, the common and tartary buckwheat hull extracts may be used as a potent source of antioxidants in mayonnaise. Since buckwheat hull extract may influence sensory attributes, colors, or physical properties of mayonnaise, when it is added to mayonnaise, additional studies are suggested to be carried out in the future.

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국문초록

단메밀과 쓴메밀 껍질 추출물에 함유된 플라보노이드 조성 및 메밀 껍질 추출물에 의한 마요네즈의 지질 산화 안정성

박봄이

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메밀은 일반적으로 도정을 하여 껍질을 제거한 알곡 형태로 사용된다. 이때 발생하는 메밀 껍질은 연 1000톤에 달하지만, 식품 소재로 활용되지 못한 채 폐기되고 있다. 그러나 메밀 껍질에는 다양한 플라보노이드가 존재하고, 이 함량이 알곡에 비해 높다고 보고되면서, 새로운 기능성 식품 소재로서 메밀 껍질의 활용과 관련한 연구가 요구되고 있다. 이에 본 연구에서는 추출 용매 종류를 달리하여 단메밀과 쓴메밀 껍질로부터 추출한 추출물의 플라보노이드 조성 및 항산화능을 비교분석하여 메밀 껍질을 기능성 식품소재로 활용할 수 있는 유용한 기초자료를 제공하고자 하였다. 또한 단메밀과 쓴메밀 껍질 추출물을 마요네즈에 적용해, 마요네즈에 함유되어 있는 지질의 천연 항산화제로서의 메밀 껍질의 활용 가능성을 확인하고자 하였다.

본 연구에서는 단메밀과 쓴메밀 껍질을 물, 에탄올(20%, 50%, 80%, 100%), 메탄올, 아세톤을 이용해 추출하였고, 각 추출물의 수율, 총 페놀 화합물 함량, 플라보노이드 조성 및 함량, 항산화능을 측정하여 비교 분석하였다. 단메밀 껍질 추출물에 함유되어 있는 플라보노이드는 vitexin, isovitexin, isoorientin, orientin, rutin, isoquercetin, quercetin이었고, 쓴메밀 껍질 추출물에서는 rutin, quercetin, isoorientin, isoquercetin이 검출되었다. 단메밀 껍질 추출물의 주요 플라보노이드는 vitexin과 isovitexin이었다. 쓴메밀 껍질 추출물의 주요 플라보노이드는 rutin과 quercetin이었으며, 단메밀 껍질 추출물에서보다 10배 이상 많았다. 단메밀과 쓴메밀 껍질의 총 플라보노이드 함량은 메탄올과 80% 에탄올로 추출한 추출물에서 가장 많았으나, 총 페놀 화합물 함량과 항산화능은 20%와 50% 에탄올을 이용한 추출물이 가장 높았다.

단메밀과 쓴메밀 껍질의 50% 에탄올 추출물(0.02% 와 0.08%)을 마요네즈에 적용하여 35℃에서 31일간 저장하면서 지질 산화 안정성을 분석하였다. 저장기간 동안 마요네즈의 PV, *p*-AV, TBA value, Totox value는 증가하였으나, 단메밀과 쓴메밀 껍질 추출물을 첨가한 마요네즈의 PV, *p*-AV, TBA value, Totox value는 대조군 및 BHT 첨가 마요네즈에 비해 낮았다. 그러나 단메밀과 쓴메밀 껍질 추출물을 첨가한 마요네즈 간의 지질 산화 안정성의 차이는 없었다. 결론적으로, 단메밀과 쓴메밀 껍질 추출물에는 페놀 화합물과 플라보노이드가 높게 함유되어 있

으며, 이 추출물을 마요네즈의 지질 산화 안정화에 활용할 수 있다고 판단한다.

주요어: 메밀껍질, 플라보노이드, 항산화능, 용매 추출, 마요네즈

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