J Food Sci Technol
https://doi.org/10.1007/s13197-019-03761-2

ORIGINAL ARTICLE

# Flavonoids in common and tartary buckwheat hull extracts and antioxidant activity of the extracts against lipids in mayonnaise

**Bom I. Park[1] · Jaecheol Kim[1] · Kiuk Lee[1] · Taehwan Lim[1] · Keum Taek Hwang[1]**

Revised: 23 March 2019 / Accepted: 28 March 2019


**Abstract** Buckwheat hulls, generally discarded as waste, have been known to possess various flavonoids and high antioxidant activities. The objective of this study was to determine effect of extracting solvents [water, ethanol (20%, 50%, 80%, and 100%), methanol, and acetone] on total phenolic content, flavonoid content and composition, and 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. The methanol and 80% ethanol extracts had more flavonoids than the others, while the aqueous ethanol extracts from both of the hulls had more total phenolics and antioxidant activities. Oxidative stability of lipids in mayonnaises added with common and tartary buckwheat hull extracts (0.02 and 0.08%, w/w) prepared by 50% ethanol were higher than that in the mayonnaise with butylated hydroxytoluene (0.02%) and control. Oxidative stability was not significantly different between the mayonnaises added with the two buckwheat hull extracts.

**Keywords** Antioxidant activity · Buckwheat hull · Flavonoid · Phenolic compound · Mayonnaise

✉ Keum Taek Hwang
keum@snu.ac.kr

[1] Department of Food and Nutrition, and Research Institute of Human Ecology, Seoul National University, Seoul 08826, Republic of Korea

## Introduction

Buckwheat, a pseudocereal which belongs to *Polygonaceae*, is consumed worldwide. Common (*Fagopyrum esculentum*) and tartary buckwheat (*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 phenolic compounds, especially flavonoids including rutin (quercetin-3-rutinoside). 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. During dehulling process, a substantial quantity of hulls is discarded as waste (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 and Yao 2007). The most commonly used solvents for extracting flavonoids from plant foods are water, aqueous ethanol, methanol, and acetone (Liu and Yao 2007). Content and composition of flavonoids in the extracts generally depend on the type of solvent, and thus their biological activity may vary. However, there have been no published data investigating influence of solvent on content and composition of flavonoids in

Published online: 10 April 2019

buckwheat hull extracts as well as their antioxidant activities.

Lipid oxidation in mayonnaise that leads to a reduction in shelf life with undesirable off-flavors is a major concern in the food industry (Gorji et al. 2016). In order to retard lipid oxidation in mayonnaise, synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and ethylenediaminetetraacetic acid (EDTA) have been commonly used (Gorji et al. 2016). However, consumers have negative impression on a chemical product (Gorji et al. 2016). Therefore, there has been a growing interest in natural antioxidants from plant materials rich in phenolic compounds. Application of 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 and Tewaruth 2010) has been studied. 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 total phenolic content, flavonoid content and composition, and 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

### Materials and chemicals

Common and tartary buckwheat hulls collected from Bongpyeong, Korea in September 2017 were obtained from Bongpyeong Agricultural Cooperative (Bongpyeong, Korea). 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 purchased from Wako Pure Chemical Co. (Osaka, Japan). Sodium thiosulfate was purchased from Junsei Chemical Co. (Tokyo, Japan). All chemicals were of analytical reagent grade.

### 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:

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

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.

### 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 mL 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 using a microplate reader (Spectramax 190, Molecular Devices, CA, USA). TPC was expressed as gallic acid equivalent (GAE).

### 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 a 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: 0–1 min, 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 an *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: 0–7 min, 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 50 °C. Detection wavelength was set at 350 nm. Five-level calibration curve was generated by analyzing known concentrations (0.25–50 μg/mL) of each standard.

### 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 microliter 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:

$$\text{DPPH or ABTS free radical scavenging activity } (\%) = (1 - \text{sample absorbance/control absorbance}) \times 100.$$

### Preparation and storage of mayonnaise

Mayonnaise was prepared with soybean oil (75%, w/w), egg yolk (9%), vinegar (6.5%), salt (1.5%), sugar (2%), and water (6%). Based on the TPC and antioxidant activities of the crude extracts, 50% ethanol extract was chosen to be applied in mayonnaise. Common or tartary buckwheat hull extract was added to the mayonnaise at 0.02 or 0.08%. Control sample was prepared without buckwheat hull extracts or BHT. A positive control was prepared adding BHT at 0.02%.

Mayonnaise was prepared as follows: all the ingredients except oil were mixed using a hand blender (Wiz Co., Daegu, Korea) for 20 s. The oil was slowly added to the mixture blending for 2 min. Mayonnaise (30 g) in a 50 mL cylindrical polystyrene tube with a screw cap was 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.

### 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 separated lipid phase was used directly for further study on oxidative stability of lipids in mayonnaise.

### Oxidative stability of lipids in mayonnaise

Progression of lipid oxidation in mayonnaise was monitored by determining peroxide value (PV), 2-thiobarbituric acid (TBA) value, and *p*-anisidine value (*p*-AV) (AOCS official method Cd 8–53, Cd 19–90, and Cd 18–90 (AOCS 2009), respectively). Total oxidation (Totox) value was calculated by 2PV + *p*-AV (Sherwin 1978).

### 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 (SPSS Inc., Chicago, IL, USA).

## Results and discussion

### Extraction yields of buckwheat hulls

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 1). Water extracted the most from both of common and tartary buckwheat hulls, while acetone did the least.

**Table 1** Extraction yields of buckwheat hulls

| 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)

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

[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 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 (613 mg GAE/g and 555 mg GAE/g in the common and tartary buckwheat hull extracts, respectively) followed by the 50% ethanol (516 mg GAE/g and 540 mg GAE/g in the common and tartary buckwheat hull extracts, respectively) and 80% ethanol extracts (404 mg GAE/g and 492 mg GAE/g in the common and tartary buckwheat hull extracts, respectively). 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. Water in the solvent may increase polarity and swell plant materials by allowing the solvent to easily penetrate the solid matrix (Singh et al. 2017), consequently an aqueous ethanol mixture having phenolic compounds be more extractable than an individual solvent.

## Identification of flavonoids in buckwheat hull extracts

HPLC–ESI–MS and HPLC–UV were used to identify major flavonoids present in the common and tartary buckwheat hull extracts. Flavonoids were identified matching the peaks observed by mass spectra and retention times with their corresponding standards. 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 (Fig. 2). Peak 1 and 2 produced *m/z* 447 of $[M–H]^-$ on MS and were identified as isoorientin and orientin, respectively. Peak 3 was determined as rutin at *m/z* 609 $[M–H]^-$. 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 with *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 epicatechin gallate. However, catechin and epicatechin gallate were not detected in the present study, but isoquercetin was.

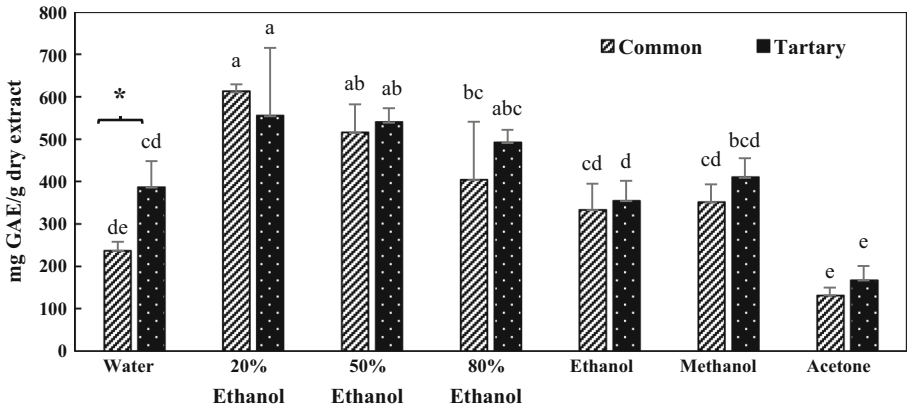


**Fig. 1** Total phenolic content in buckwheat hull extracts prepared by various solvents. *GAE*, gallic acid equivalent. Values are means ± standard deviations (n = 3). [a,b,c,d,e]Different letters indicate significant differences within the same buckwheat hull extracts ($p < 0.05$; one-way ANOVA and Duncan's multiple range test). *Significant difference between common and tartary buckwheat hull extracts ($p < 0.05$; independent t-test)

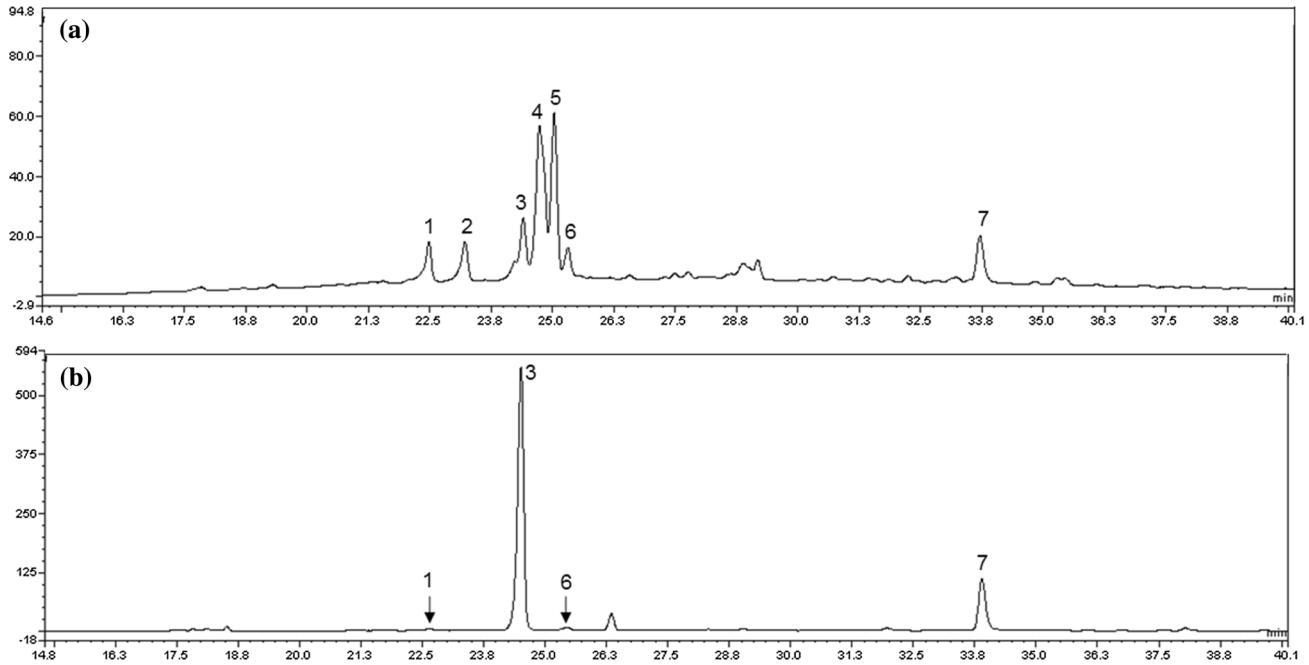


**Fig. 2** HPLC chromatograms analyzing flavonoids in (**a**) common and (**b**) tartary buckwheat hull extracts (λ = 350 nm). The peaks represent 1, isoorientin; 2, orientin; 3, rutin; 4, isovitexin; 5, vitexin; 6, isoquercetin; and 7, quercetin

## Composition of flavonoids in buckwheat hull extracts

Individual flavonoids in the common and tartary buckwheat hull extracts were quantified using their corresponding standards. As shown in Table 2, the common buckwheat hull extracts contained more diverse flavonoids than the tartary buckwheat hull extracts. 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 in the common buckwheat hull extracts than rutin. Lee et al. (2016) reported that rutin is the major flavonoid in both common and tartary buckwheat hulls. However, Zhang et al. (2017), who identified the major flavonoids in eight cultivars, reported that vitexin and isovitexin are the most abundant flavonoids in the six cultivars of common buckwheat hulls. 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), and anti-tumor (Choi et al. 2006) activities. Vitexin, isovitexin, and rutin were highly extracted when using methanol (12.2 mg/g, 7.36 mg/g, and 5.37 mg/g, respectively).

Isoorientin and orientin have been reported to exist only in buckwheat hulls, but not in groats (Lee et al. 2016). In this study, both of isoorientin and orientin were detected in common buckwheat hull extracts with the highest in the 50% ethanol extract (1.35 mg/g 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, 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 hull contains various flavonoids, while buckwheat groat only contains rutin and isovitexin (Krahl et al. 2008). In this study, various functional flavonoids were 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 2). 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 content 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). Bhinder et al. (2019) reported that rutin was the major flavonoid present largely in the free form and more tolerant to thermal treatment than isovitexin, vitexin, and orientin.

Quercetin, the second largest flavonoid in the tartary buckwheat hull extracts, was more detected in the

**Table 2** Composition of major flavonoids in buckwheat hull extracts

| Solvent | Content (mg/g dry extract) | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | Isoorientin | Orientin | Rutin | Isovitexin | Vitexin | Isoquercetin | Quercetin | |
| *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$^{a}$ | 12.2 ± 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 | 43.1 ± 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 | 53.4 ± 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 | 60.0 ± 3.29$^{ab}$ | ND | ND | 3.76 ± 0.33$^{a}$ | 15.0 ± 1.03$^{c}$ | 79.7 ± 4.4$^{b}$ |
| 80% Ethanol | 0.32 ± 0.15$^{b}$ | ND | 68.5 ± 9.45$^{a}$ | ND | ND | 1.98 ± 0.60$^{b}$ | 20.3 ± 2.23$^{b}$ | 91.1 ± 11.0$^{ab}$ |
| Ethanol | ND | ND | 36.9 ± 9.10$^{c}$ | ND | ND | 1.96 ± 0.77$^{b}$ | 27.9 ± 3.05$^{a}$ | 66.8 ± 11.5$^{c}$ |
| Methanol | ND | ND | 66.0 ± 5.33$^{a}$ | ND | ND | 3.36 ± 0.83$^{a}$ | 28.2 ± 2.07$^{a}$ | 97.5 ± 6.9$^{a}$ |
| Acetone | ND | ND | 7.80 ± 2.19$^{d}$ | ND | ND | 1.51 ± 0.16$^{b}$ | 11.7 ± 2.84$^{c}$ | 21.0 ± 5.1$^{e}$ |

Values are means ± standard deviations (n = 3)

*ND*, not detected

$^{a,b,c,d,e,f}$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)

methanol and ethanol extracts (28.2 mg/g 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 Rupasinghe et al. (2011), who observed that quercetin was not detected in water extract, but only in methanol and acetone extracts. As with the results of rutin, quercetin was much more in the tartary buckwheat hull extracts 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 detected in large amounts in the 50% ethanol extracts (1.88 mg/g and 3.76 mg/g for the common and tartary buckwheat hull extracts, respectively). Zhang et al. (2011) reported that isoquercetin has a regulative role in blood glucose level and lipids.

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 extracted the most amount of flavonoids (29.3 mg/g and 97.5 mg/g in the common and tartary buckwheat hull extracts, respectively) followed by 80% ethanol (24.8 mg/g and 91.1 mg/g in the common and tartary buckwheat hull extracts, respectively) and 50% ethanol (22.9 mg/g and 79.7 mg/g in the common and tartary buckwheat hull extracts, respectively). Acetone was the poorest solvent for extracting flavonoids (2.5 mg/g and 21.0 mg/g in the common and tartary buckwheat hull extracts, respectively).

The extraction solvents affect flavonoid content and composition of buckwheat hull extracts. In this study common and tartary buckwheat hulls contained flavonoids more soluble in methanol and 80% ethanol than in the other solvents. The differences in the flavonoid content and composition of the extracts by various solvents could be attributed to their polarities. However, even though methanol and acetone are similar in polarity, acetone extracted less flavonoids than methanol. It could be

attributed to the fact that acetone is an aprotic solvent, which is not a hydrogen bond donor. Galanakis et al. (2013) also mentioned that phenolic compounds including flavonoids prefer polar protic solvent (methanol or ethanol) to polar aprotic solvent (acetone). Thus, extraction of flavonoids from buckwheat hull extracts might be affected by polarity of solvents as well as their intermolecular interactions.

### Antioxidant activities of buckwheat hull extracts

Extracting solvents also affected antioxidant activities of the buckwheat hull extracts (Table 3). Both 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. 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 with organic solvents than water, while polyphenol yield and antioxidant properties were significantly higher in water extract. The results suggest that other phenolic compounds, which were not identified in this study, may also contribute to antioxidant activity.

### Oxidative stability of lipids in mayonnaise

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. Oxidative stability of lipids in mayonnaise was determined under an accelerated oxidation condition at 35 °C during storage for 31 days. PV of lipids in the mayonnaise added with the buckwheat hull extracts were considerably lower than those of the control and BHT-added mayonnaise during storage (Fig. 3). PV of lipids in the mayonnaises were initially 0.5–0.6 meq/kg and gradually increased. However, the control mayonnaise reached the highest PV (98.3 meq/kg) by the 26th day and decreased to 84.1 meq/kg on the 31st day. This could be due to degradation of peroxides into secondary oxidative products (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 the 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, suggesting that well known major flavonoids in buckwheat hulls such as rutin and quercetin may contribute to retardation of lipid oxidation in mayonnaise.

TBA values of all the mayonnaise samples continuously increased during storage (Fig. 3), 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 the buckwheat hull extracts were lower than those of the control and BHT-added mayonnaise. By the 31st day, the control showed the highest TBA value (0.1 $mg^{-1}$), while the mayonnaise added with the tartary buckwheat hull extracts (0.08%) showed the lowest TBA value (0.047 $mg^{-1}$).

**Table 3** Antioxidant activities of buckwheat hull extracts

| Solvent | DPPH radical scavenging activity (%) | | ABTS radical scavenging activity (%) | |
|---|---|---|---|---|
| | Common | Tartary | Common | Tartary |
| Water | $67.7 \pm 2.81^{b}$ | $72.1 \pm 5.12^{b}$ | $75.9 \pm 5.4^{b}$ | $77.6 \pm 1.88^{c}$ |
| 20% ethanol | $86.5 \pm 2.88^{a}$ | $81.8 \pm 2.73^{a}$ | $95.4 \pm 0.6^{a}$ | $90.3 \pm 2.06^{a}$ |
| 50% ethanol | $85.8 \pm 1.55^{a}$ | $82.0 \pm 2.14^{a}$ | $95.4 \pm 0.9^{a}$ | $85.9 \pm 1.99^{b}$ |
| 80% ethanol | $71.3 \pm 0.99^{b}$ | $72.2 \pm 4.31^{b}$ | $92.2 \pm 1.0^{a}$ | $83.8 \pm 3.04^{b}$ |
| Ethanol | $39.9 \pm 2.95^{d}$ | $38.7 \pm 3.29^{d}$ | $74.0 \pm 3.1^{b}$ | $65.1 \pm 0.69^{d}$ |
| Methanol | $57.1 \pm 5.98^{c}$ | $50.3 \pm 4.34^{c}$ | $74.1 \pm 2.7^{b}$ | $67.1 \pm 1.62^{d}$ |
| Acetone | $23.4 \pm 2.01^{e}$ | $8.92 \pm 1.88^{e}$ | $21.0 \pm 4.1^{c}$ | $32.6 \pm 2.65^{e}$ |

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)

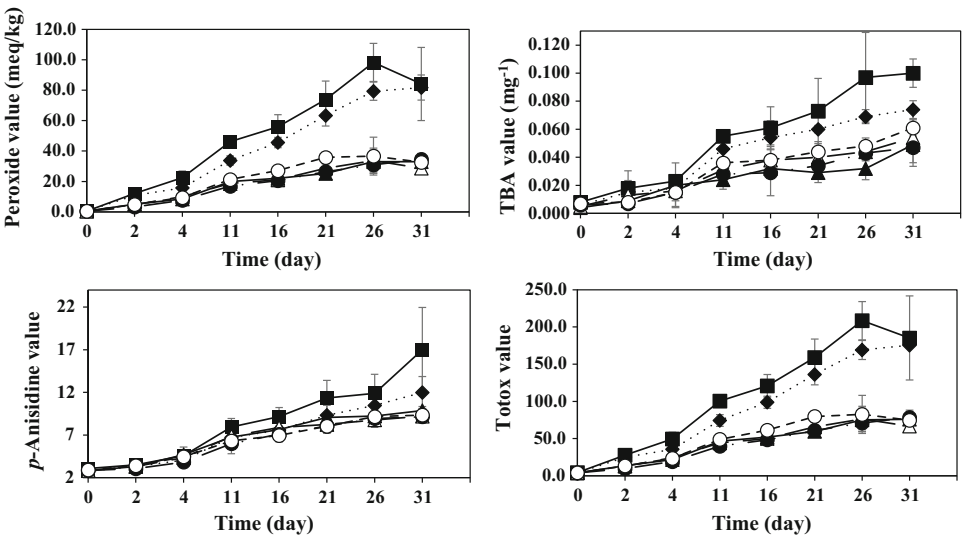


**Fig. 3** Oxidation levels in the lipid phase of mayonnaise during storage at 35 °C for 31 days. Values are means ± standard deviations (n = 3). ■ control, ◆ added with 0.02% butylated hydroxytoluene, ▲ added with 0.08% common buckwheat hull extract powder, △ added with 0.02% common buckwheat hull extract powder, ● added with 0.08% tartary buckwheat hull extract powder, ○ added with 0.02% tartary buckwheat hull extract powder

*p*-AV of lipids in all the mayonnaises gradually increased with storage time (Fig. 3). *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 resulting 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 delayed the development of secondary oxidation products. The lowest *p*-AV (9.14) was observed in the mayonnaise added with the tartary buckwheat hull extract at 0.08% as with the result of TBA on the 31st day. The highest *p*-AV (17.0) was observed in the control on the 31st day. However, there was no significant difference in *p*-AV among the mayonnaises added with BHT and buckwheat hull extracts. Moreover, the added levels of buckwheat hull extracts did not significantly affect *p*-AV.

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 (Fig. 3). 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 or 0.08% common or tartary buckwheat hull extract in mayonnaise could more effectively retard lipid oxidation than that of BHT. Li et al. (2014) reported that purple corn husk extract-added mayonnaise demonstrated lower PV, *p*-AV, Totox value than BHT-added mayonnaise.

Since both of the common and tartary buckwheat hull extracts prepared by 50% ethanol retarded the lipid oxidation in mayonnaise compared with BHT, they might be considered to be an effective antioxidant to the lipids in mayonnaise.

## Conclusions

Types of extracting solvents considerably affected yield, TPC, and flavonoid content and composition as well as antioxidant activities of common and tartary buckwheat hull extracts. 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. Regardless of the type of solvent, vitexin was the most abundant in common buckwheat hull extracts, while rutin was in tartary buckwheat hull extracts. Addition of common or tartary buckwheat hull extract efficiently increased oxidative stability of lipids in mayonnaise when PV, TBA, *p*-AV, and Totox value were determined. Moreover, in comparison with BHT, buckwheat hull extracts more effectively delayed lipid oxidation in mayonnaise. Therefore, the common and tartary buckwheat hull extracts using 50% ethanol could be applied in food matrix, because they are rich in phenolic compounds with various flavonoids and high in antioxidant activities as well. Further studies are suggested to be carried out in the future, since buckwheat hull extract may influence sensory attributes, colors, or physical properties of mayonnaise, when it is added to mayonnaise.

**Acknowledgements** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Authors' contributions** BIP conceived and designed the study and KTH organized the whole research outline. All experimental works were conducted and the manuscript was written by BIP. JK assisted extraction of buckwheat hull extracts and HPLC–UV analysis. KL offered scientific advices on the LC–MS. TL offered scientific advices on mayonnaise oxidation study. Manuscript drafting was corrected by KTH and BIP. All authors have approved and reviewed the final manuscript.

## References


Altunkaya A, Hedegaard RV, Harholt J, Brimer L, Gökmen V, Skibsted LH (2013) Oxidative stability and chemical safety of mayonnaise enriched with grape seed extract. Food Funct 4:1647–1653

AOCS (2009) Official methods and recommended practices of the American Oil Chemists' Society. AOCS Press, Urbana

Bhinder S, Singh B, Kaur A, Singh N, Kaur M, Kumari S, Yadav MP (2019) Effect of infrared roasting on antioxidant activity, phenolic composition and Maillard reaction products of tartary buckwheat varieties. Food Chem 285:240–251

Borghi SM, Carvalho TT, Staurengo-Ferrari L, Hohmann MS, Pinge-Filho P, Casagrande R, Verri WA Jr (2013) Vitexin inhibits inflammatory pain in mice by targeting TRPV1, oxidative stress, and cytokines. J Nat Prod 76:1141–1149

Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 28:25–30

Choi HJ, Eun JS, Kim BG, Kim SY, Jeon H, Soh Y (2006) Vitexin, an HIF-1α inhibitor, has anti-metastatic potential in PC12 Cells. Mol Cells 22:291–299

Dziadek K, Kopeć A, Pastucha E, Piątkowska E, Leszczyńska T, Pisulewska E, Witkowicz R, Francik R (2016) Basic chemical composition and bioactive compounds content in selected cultivars of buckwheat whole seeds, dehulled seeds and hulls. J Cereal Sci 69:1–8

Galanakis CM, Goulas V, Tsakona S, Manganaris GA, Gekas V (2013) A knowledge base for the recovery of natural phenols with different solvents. Int J Food Prop 16:382–396

Gorji SG, Smyth HE, Sharma M, Fitzgerald M (2016) Lipid oxidation in mayonnaise and the role of natural antioxidants: a review. Trends Food Sci Technol 56:88–102

Guo XD, Wu CS, Ma YJ, Parry J, Xu YY, Liu H, Wang M (2012) Comparison of milling fractions of tartary buckwheat for their phenolics and antioxidant properties. Food Res Int 49:53–59

Holasova M, Fiedlerova V, Smrcinova H, Orsak M, Lachman J, Vavreinova S (2002) Buckwheat—the source of antioxidant activity in functional foods. Food Res Int 35:207–211

Kim YS, Lee JH (2017) Effects of hydrolyzed rapeseed cake extract on the quality characteristics of mayonnaise dressing. J Food Sci 82:2847–2856

Kim JH, Lee BC, Kim JH, Sim GS, Lee DH, Lee KE, Yun YP, Pyo HB (2005) The isolation and antioxidative effects of vitexin from *Acer palmatum*. Arch Pharm Res 28:195

Krahl M, Back W, Zarnkow M, Kreisz S (2008) Determination of optimised malting conditions for the enrichment of rutin, vitexin and orientin in common buckwheat (*Fagopyrum esculentum* Moench). J Inst Brew 114:294–299

Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids: an overview. Sci World J 2013:1–16

Lagunes-Galvez L, Cuvelier ME, Ordonnaud C, Berset C (2002) Oxidative stability of some mayonnaise formulations during storage and daylight irradiation. J Food Lipids 9:211–224

Lapornik B, Prošek M, Wondra AG (2005) Comparison of extracts prepared from plant by-products using different solvents and extraction time. J Food Eng 71:214–222

Lee LS, Choi EJ, Kim CH, Sung JM, Kim YB, Seo DH, Choi HW, Choi YS, Kum JS, Park JD (2016) Contribution of flavonoids to the antioxidant properties of common and tartary buckwheat. J Cereal Sci 68:181–186

Li CY, Kim HW, Li H, Lee DC, Rhee HI (2014) Antioxidative effect of purple corn extracts during storage of mayonnaise. Food Chem 152:592–596

Liu Q, Yao H (2007) Antioxidant activities of barley seeds extracts. Food Chem 102:732–737

Lu L, Murphy K, Baik BK (2013) Genotypic variation in nutritional composition of buckwheat groats and husks. Cereal Chem 90:132–137

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26:1231–1237

Sedej I, Sakač M, Mandić A, Mišan A, Tumbas V, Čanadanović-Brunet J (2012) Buckwheat (*Fagopyrum esculentum* Moench) grain and fractions: antioxidant compounds and activities. J Food Sci 77:C954–C959

Sherwin ER (1978) Oxidation and antioxidants in fat and oil processing. J Am Oil Chem Soc 55:809–814

Singh B, Singh N, Thakur S, Kaur A (2017) Ultrasound assisted extraction of polyphenols and their distribution in whole mung bean, hull and cotyledon. J Food Sci Technol 54:921–932

Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods Enzymol 299:152–178

Tananuwong K, Tewaruth W (2010) Extraction and application of antioxidants from black glutinous rice. LWT-Food Sci Technol 43:476–481

Vasantha Rupasinghe HP, Kathirvel P, Huber GM (2011) Ultrasonication-assisted solvent extraction of quercetin glycosides from 'Idared' apple peels. Molecules 16:9783–9791

Vrinda B, Devi PU (2001) Radiation protection of human lymphocyte chromosomes in vitro by orientin and vicenin. Mutat Res Genet Toxicol Environ Mutagen 498:39–46

Vuong QV, Hirun S, Roach PD, Bowyer MC, Phillips PA, Scarlett CJ (2013) Effect of extraction conditions on total phenolic compounds and antioxidant activities of *Carica papaya* leaf aqueous extracts. J Herb Med 3:104–111

Yi BR, Ka HJ, Kwon YJ, Choi HS, Kim SH, Kim J, Lee MJ (2017) Oxidative stability in oil-in-water emulsions with quercetin or rutin under iron catalysis or riboflavin photosensitization. J Food Sci 82:890–896

Yilmaz Y, Toledo RT (2006) Oxygen radical absorbance capacities of grape/wine industry byproducts and effect of solvent type on extraction of grape seed polyphenols. J Food Compos Anal 19:41–48

Yuan L, Wang J, Xiao H, Xiao C, Wang Y, Liu X (2012) Isoorientin induces apoptosis through mitochondrial dysfunction and inhibition of PI3K/Akt signaling pathway in HepG2 cancer cells. Toxicol Appl Pharmacol 265:83–92

Zhang R, Yao Y, Wang Y, Ren G (2011) Antidiabetic activity of isoquercetin in diabetic KK-$A^y$ mice. Nutr Metab 8:85

Zhang ZL, Zhou ML, Tang Y, Li FL, Tang YX, Shao JR, Xue WT, Wu YM (2012) Bioactive compounds in functional buckwheat food. Food Res Int 49:389–395

Zhang W, Zhu Y, Liu Q, Bao J, Liu Q (2017) Identification and quantification of polyphenols in hull, bran and endosperm of common buckwheat (*Fagopyrum esculentum*) seeds. J Funct Food 38:363–369