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Full Length Research Paper

Evaluation of phytonutrients in Adlay (*Coix lacryma-jobi* L.) seeds

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Adlay (*Coix lacryma-jobi* L.) has long been cultivated in oriental countries as a cereal and medicinal crop. In this study, eight adlay varieties, most commonly cultivated in South Korea were selected and their seed phytonutrient contents were evaluated. The average total vitamin E content was 37.38 mg kg^{-1}. Two major components of vitamin E were γ-tocopherol (14.76 mg kg^{-1}) and γ-tocotrienol (14.18 mg kg^{-1}), corresponding to 39.5 and 37.9% of the total vitamin E content, respectively. Tested varieties exhibited squalene content ranging from 31.23 to 55.00 mg kg^{-1}, with an average of 41.24 mg kg^{-1}. The contents of the phytosterols: Campesterol, stigmasterol, and β-sitosterol were 80.43, 131.1, and 313.38 mg kg^{-1}, respectively. Of the six detected fatty acids, the major fatty acids were oleic (46.3%) and linoleic acids (37.4%). Seed squalene content exhibited high correlationship with phytosterols, but not with tocopherols. Among the tested varieties, variety Yulmu 1 showed a relatively higher proportion of phytonutrients, suggesting its potential as a gene source for further breeding.

Key words: Adlay, vitamin E, phytosterols, squalene, fatty acids.

INTRODUCTION

Adlay (*Coix lacryma-jobi* L), also called soft-shelled Job's tears or Chinese pearl barley) is an annual grass crop that has long been used as a traditional medicine and a nourishing food in oriental countries. It is widely cultivated in China, Taiwan, Japan, Thailand and Korea. Adlay seeds have been used to treat warts, chapped skin, rheumatism and neuralgia, and it is an anti-inflammatory and anthelmintic agent (Hsu et al., 2003). Modern medical research has revealed anti-proliferative, anticancer (Chang et al., 2003; Lee et al., 2008) and anti-allergic (Hsu et al., 2003) activities in adlay seeds. Adlay seeds were found to suppress early events in colon carcinogenesis (Shih at al., 2004), beneficial to cardiovascular and intestinal health (Wang et al., 2011) and have blood lipid-reducing and antioxidant effects (Yu et al., 2011). In addition, a number of benzoxazinones in adlay seed s exhibit anti-inflammatory activity (Nagao et al., 1985; Huang et al., 2009), and the extracts of adlay seeds are effective against viral infections (Hidaka et al., 1992), and can be used as therapeutic and functional food for obesity patients (Ha et al., 2010). It also have hypolipidemic (Kim et al., 2004) and antimutagenic effects.

Adlay has also been cultivated as a cereal crop and may contain various nutraceutical compounds such as vitamin E, phytosterols and squalene (Park et al., 2004; Wu et al., 2007). Vitamin E, consisting of α-, β-, γ- and δ-tocopherols and the corresponding tocotrienols (α-, β-, γ- and δ-tocotrienols), is a group of lipid soluble compounds. Tocopherols have been well documented as natural antioxidants, and among the four isomers, α-tocopherol is the most biologically active form (Ohkatsu et al., 2001). However, α-tocotrienol has more than three times the efficiency as a scavenger of peroxyl radicals (Packer, 1995) compared to α-tocopherol. Tocotrienols (T3) exhibit additional health-beneficial effects, such as antioxidative, antiproliferative (Choi and Lee, 2009), anticancer (Wada et al., 2005) and cholesterol-lowering effects. Although, vitamin E isomer content in adlay seeds has been studied

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(Park et al., 2004), only one variety had been selected and the variety name was not clearly identified. Similar to various cereal crops, the unsaponifiable fraction of adlay seeds may contain various beneficial compounds including squalene, a hydrocarbon used in cholesterol biosynthesis (Moreda et al., 2001) that may possess tumor proliferative and serum cholesterol-lowering effects (Khor and Chieng, 1997), and phytosterols, cholesterol-like compounds with serum-cholesterol lowering effects (Marangoni and Poli, 2010). A preventive effect on colon cancer development (Awad and Fink, 2000) and lung cancer protective effects have also been ascribed to adlay seeds (Mendilaharsu et al., 1998). Adlay seeds contain 7.0% lipids (Wu et al., 2007), which are composed of various fatty acids that influence human physiological and biochemical processes (Nageswari et al., 1999; Borsoleno and Galduroz, 2008). A diet rich in monounsaturated fatty acids, such as oleic acid, may decrease blood cholesterol levels (Hargrove et al., 2001) and improve HDL fluidity (Villa et al., 2002). Similarly, polyunsaturated fatty acids such as linoleic and linolenic acids may benefit the structure and function of membrane proteins, enzymes, and active transport molecules (Yaqoob, 2002). However, few details on the fatty acids in adlay seeds have been reported. This study focused on identifying the vitamin E, squalene and phytosterol contents, and fatty acid composition in seeds of the eight adlay varieties that are most widely cultivated in South Korea.

MATERIALS AND METHODS

Plant materials

Eight varieties most popularly cultivated in South Korea were selected and cultivated in Yoncheon, South Korea, according to standard cultivation practice protocols provided by the Rural Development Administration of Korea. The unpolished seeds of each variety were ground and stored at 4°C under airtight conditions until analysis.

Sample preparation for vitamin E, squalene and phytosterol analyses

Samples for vitamin E and phytosterol analyses were prepared based on the method of Park et al. (2004) with some modifications. Powdered adlay samples (1.0 g) were placed in a 50 ml tube, and 0.1 g ascorbic acid and 10 ml ethanol were added and shaken in a water bath at 80°C for 10 min. Then, 300 µl of 44% KOH was added and the mixture was shaken for saponification for 18 min under the same conditions. The tubes were cooled rapidly in an ice bucket; 10 ml n-hexane and 10 ml of distilled water were added, mixed, and centrifuged at 1900 x g for 10 min; and the upper hexane layer was collected. This process was repeated three times, and the collected hexane layer was washed three times with 10 ml distilled water and passed through anhydrous Na_2SO_4 to remove water, concentrated on a rotary evaporator, dissolved in 1 ml of isooctane, and filtered through a 0.45 µm nylon syringe filter. The solution was injected into a high performance liquid chromatography (HPLC) system to analyze vitamin E content or into a gas chromatography (GC) system for phytosterol and squalene analysis.

Vitamin E analysis

Eight vitamin E isomers (α-, β-, γ- and δ- tocopherols, and α-, β-, γ- and δ- tocotrienols) were quantified by HPLC (Sykam, S1101, Gilchingen, Germany) following Park et al. (2004). HPLC was performed with a Supelcosil LC-Si (5 µm, 250 × 46 mm ID) column and a fluorescence detector with excitation and emission at 290 and 330 nm, respectively. The mobile phase was isooctane: ethyl acetate: acetic acid: 2,2,-dimethoxy propane = 98.5: 0.7: 0.7: 0.1, and the flow rate was 1.5 ml min^{-1}. The injection volume was 20 µl, and the peaks were identified by comparison to the retention times of authentic standards.

Squalene and phytosterol analysis

Squalene, campesterol, stigmasterol and β-sitosterol contents were determined by GC (Varian 3800, Palo Alto, CA, USA). This analysis was performed with a capillary column (CP-SIL 8CB, 30 × 0.25 mm, 0.4 µm film thickness) with the injector and flame ionization detector temperatures set at 290°C. The injection volume was 1 µl with a split ratio of 1:20 and a constant column flow (1.0 ml min^{-1}) of helium gas. The oven temperature was initially set at 220°C for 2 min, increased to 290°C by 5°C min^{-1}, held for 14 min, and then increased to 310°C at a rate of 10°C min^{-1}. Squalene and phytosterol peaks were identified by comparison to the retention time of authentic standard peaks.

Sample preparation for fatty acid composition

Samples for fatty acid composition analysis were prepared according to Kim et al. (2000). Powdered samples (0.5 g) were mixed with 680 µl of a methylation mixture (MeOH: benzene: 2,2-dimethoxypropane: H_2SO_4 = 39: 20: 5: 2) and 400 µl of heptane. After vigorous mixing, the solution was heated for 2 h at 80°C in a water bath and cooled to room temperature. The heptane layer was collected by centrifugation and was injected into the GC for fatty acid composition analysis.

Fatty acid composition analysis

Fatty acid composition was analyzed according to Kim et al. (2000) using a capillary column (CP-SIL 88 CB FAME, 50 × 0.25 mm, 0.2 µm film thickness; Supelco, Bellefonte, PA, USA). The injector and detector temperature was 210°C, with He as the carrier gas. Injection volume was 1 µl with a split ratio of 1:50 on constant column flow (1.0 ml min^{-1}). Oven temperature was initially set at 110°C for 5 min, increased to 210°C at 5°C min^{-1}, and held for 40 min. A mixture of 37 FAME standards (Supelco) was used to identify the peaks.

Standard solution preparation

Eight vitamin E isomers were prepared by dissolving them separately in iso-octane to prepare 1000 ppm stock solutions. Later, they were mixed and diluted to different concentrations. Similarly, squalene and phytosterol standards were dissolved in chloroform to prepare different concentrations. A mixture of 37 FAME standards was diluted to the desired concentration with methylene chloride.

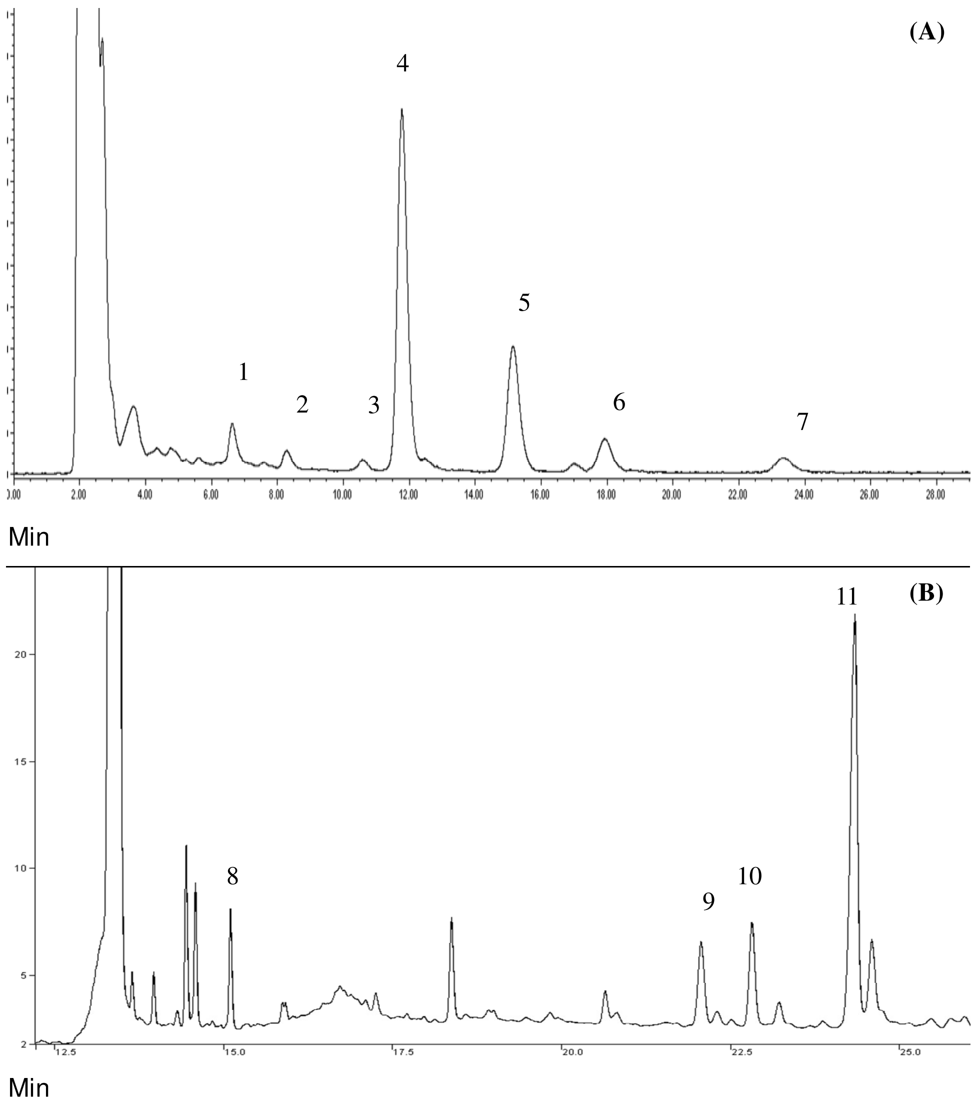

Figure 1. High performance liquid chromatogram showing vitamin E isomers (A) and gas chromatogram showing squalene and phytosterols (B) in adlay seeds. Peak identification, 1: α-tocopherol, 2: α-tocotrienol, 3: β-tocopherol, 4: γ-tocopherol, 5: γ- tocotrienol, 6: δ- tocopherol, 7: δ- tocotrienol, 8: squalene, 9: campesterol, 10: stigmasterol and 11: β-sitosterol.

Chemicals and reagents

Vitamin E standards (tocopherols: α-T, β-T, γ-T and δ-T; and tocotrienols: α-T3, β-T3, γ-T3 and δ-T3) were obtained from Merck (Darmstadt, Germany). Authentic standards for squalene, stigmasterol, β-sitosterol, and campesterol were purchased from Sigma-Aldrich (St. Louis, MO, USA), and 37 FAME from Supleco (USA). Ascorbic acid and anhydrous sodium sulfate were obtained from Samchun (Seoul, Republic of Korea) and potassium hydroxide was purchased from Dae Jung (Seoul, Republic of Korea). All solvents and chemicals used were of HPLC grade.

Data analysis

Means of three independent sample replications were used and statistical analyses were performed with Duncan's multiple range test using SPSS version 18 (SPSS, Inc., Chicago, IL, USA) at a significance level of 0.05.

RESULTS AND DISCUSSION

Vitamin E content

Seven isomers of vitamin E (four tocopherols and three tocotrienols) were detected under our experimental conditions (Figure 1A). In all tested adlay varieties, the dominant vitamin E isomers were γ-tocopherol (14.76 mg kg^{-1}) and γ-tocotrienol (14.18 mg kg^{-1}), representing 39.5 and 37.9% of the total, respectively (Table 1). Total tocopherol contents varied from 15.20 (variety, Sanggang) to 23.30 mg kg^{-1} (variety Yulmu 1) with an

Table 1. Tocopherol and tocotrienol contents in adlay seeds (mg kg^{-1}).

Variety	Tocopherol (T)					Tocotrienol (T3)				Total vitamin E
	α-T	β-T	γ-T	δ-T	Total T	α-T3	γ-T3	δ-T3	Total T3	
Aewon	1.76^{a*}	1.13^{cd}	15.95^{ab}	2.14^{ab}	20.98^{ab}	2.20^{a}	11.72^{a}	1.50^{a}	15.42^{a}	36.40^{ab}
Daechong	1.96^{a}	1.32^{d}	13.17^{ab}	1.68^{ab}	18.14^{ab}	1.47^{a}	14.37^{a}	1.79^{a}	17.63^{ab}	35.77^{ab}
Johyun	1.64^{a}	1.05^{c}	13.29^{ab}	2.08^{ab}	18.07^{ab}	1.76^{a}	10.34^{a}	1.55^{a}	13.65^{a}	31.72^{a}
Kimjejong	1.83^{a}	0.74^{a}	15.38^{ab}	1.69^{ab}	19.64^{ab}	2.36^{a}	15.20^{a}	1.47^{a}	19.02^{ab}	38.66^{ab}
Pungseongyulmu	1.71^{a}	1.39^{d}	15.45^{ab}	1.97^{ab}	20.52^{ab}	2.15^{a}	14.40^{a}	1.77^{a}	18.32^{ab}	38.84^{ab}
Sanggang	1.66^{a}	0.93^{abc}	11.20^{a}	1.42^{a}	15.20^{a}	1.52^{a}	13.43^{a}	1.78^{a}	16.73^{a}	31.93^{a}
Suwon 3	1.65^{a}	0.97^{abc}	15.66^{ab}	1.81^{ab}	20.09^{ab}	2.52^{a}	14.12^{a}	1.73^{a}	18.37^{ab}	38.46^{ab}
Yulmu 1	2.05^{a}	0.81^{ab}	17.98^{b}	2.46^{b}	23.30^{b}	1.93^{a}	19.87^{b}	2.16^{b}	23.96^{b}	47.25^{b}
Average	1.78	1.04	14.76	1.91	19.49	1.99	14.18	1.72	17.89	37.38

*Values are mean of three replications. Means followed by different letters within a column are significantly different from each other at $p < 0.05$ by Duncan's multiple range test.

average of 19.49 mg kg^{-1}. This present study shows similar results in terms of total tocopherol contents as studied earlier (18.0 mg kg^{-1}) by Lee et al. (2008). Total tocotrienol contents varied from from 13.65 (variety Johyun) to 23.96 mg kg^{-1} (variety Yulmu 1) with an average of 17.89 mg kg^{-1}. Total vitamin E content varied from 31.72 (var. Johyun) to 47.25 mg kg^{-1} (variety, Yulmu 1) with an average of 37.38 mg kg^{-1}, similar to Wu et al. (2007) finding of 36.4 to 51.9 mg kg^{-1} total vitamin E content in polished adlay seeds. The presence of the highest vitamin E content in variety, Yulmu 1, suggested its potential use as a gene source for breeding.

Squalene content

Squalene content in the eight tested adlay seeds ranged from 31.23 (var. Kimjejong) to 55.00 mg kg^{-1} (var. Yulmu 1) (Table 2), and the average squalene content was 41.24 mg kg^{-1}. Similar results were reported by Wu et al. (2007), who found 41.0 to 61.2 mg kg^{-1} squalene in polished adlay seeds collected from four different countries. This present study shows a relatively higher squalene content compared to that in barley (2.8), buckwheat (24.8), maize (22.0), and spelt (26.8 mg kg^{-1}) (Ryan et al., 2007). Our results reveal that adlay seeds can be used as an alternative source of cereal in terms of squalene content.

Phytosterol content

We analyzed three phytosterols and squalene by GC (Figure 1B). The average contents of campesterol, stigmasterol and β-sitosterol were 80.43, 131.07 and 313.38 mg kg^{-1}, respectively (Table 2). Total phytosterol contents in the tested varieties ranged from 457.06 (var. Johyun) to 626.89 mg kg^{-1} (var. Yulmu 1) with an average of 524.88 mg kg^{-1}. Wu et al. (2007) also reported similar levels for campesterol (58 to 106 mg kg^{-1}) and β-sitosterol (232 to 638 mg kg^{-1}) contents in polished adlay seeds, whereas stigmasterol content (53.4 to 88.8 mg kg^{-1}) was much lower than our findings (106.93 to 62.73 mg kg^{-1}), which may be due to genetic differences. Compared to other cereals, such as oats at 447 (Piironen et al., 2002), sorghum at 500 (Singh et al., 2003), barley at 525, and maize at 452.0 mg kg^{-1} (Ryan et al., 2007), adlay seeds contained higher total phytosterols (524.88 mg kg^{-1}). Thus, adlay seeds could be better in terms of phytosterol intake than other grains.

Table 2. Squalene and phytosterol contents in adlay seeds (mg kg^{-1}).

Variety	Squalene	Campesterol	Stigmasterol	β-Sitosterol	Total phytosterol
Aewon	43.73^{ab*}	79.56^{ab}	130.20^{ab}	305.96^{abc}	515.72^{abc}
Daechong	42.47^{ab}	83.83^{ab}	131.80^{ab}	315.40^{abc}	531.03^{abc}
Johyun	31.33^{a}	72.53^{a}	120.80^{ab}	263.73^{a}	457.06^{a}
Kimjejong	31.23^{a}	70.73^{a}	106.93^{a}	284.76^{ab}	462.42^{a}
Pungseongyulmu	45.83^{ab}	92.73^{b}	143.23^{bc}	361.66^{bc}	597.62^{bc}
Sanggang	43.60^{ab}	81.17^{ab}	138.76^{bc}	306.33^{abc}	526.26^{abc}
Suwon 3	36.70^{a}	75.03^{ab}	114.13^{ab}	292.96^{ab}	482.12^{ab}
Yulmu 1	55.00^{b}	87.86^{ab}	162.73^{c}	376.23^{c}	626.82^{bc}
Average	41.24	80.43	131.1	313.38	524.88

*Values are mean of three replications. Means followed by different letters within a column are significantly different to each other at $p < 0.05$ by Duncan's multiple range test.

Table 3. Fatty acid composition of adlay seeds (%).

Variety	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Arachidic (C20:0)
Aewon	13.3^{a*}	1.8^{a}	49.3^{c}	34.7^{a}	0.7^{a}	0.3^{a}
Daechong	13.4^{a}	2.1^{abc}	47.9^{c}	35.7^{b}	0.6^{a}	0.3^{a}
Johyun	13.6^{a}	2.4^{c}	45.3^{ab}	37.9^{cd}	0.5^{a}	0.3^{a}
Kimjejong	13.7^{a}	2.0^{ab}	46.1^{b}	37.5^{cd}	0.6^{a}	0.2^{a}
Pungseongyulmu	12.8^{a}	2.3^{bc}	43.8^{a}	40.3^{e}	0.8^{a}	0.1^{a}
Sanggang	12.9^{a}	2.3^{bc}	45.8^{b}	38.1^{d}	0.7^{a}	0.3^{a}
Suwon 3	13.2^{a}	1.9^{ab}	46.0^{b}	38.0^{cd}	0.6^{a}	0.3^{a}
Yulmu 1	13.4^{a}	2.1^{abc}	46.4^{b}	37.2^{c}	0.7^{a}	0.2^{a}
Average	13.4	2.1	46.3	37.4	0.6	0.3

*Values are mean of three replications. Means followed by different letters within a column are significantly different to each other at $p < 0.05$ by Duncan's multiple range test.

Fatty acid composition

Among the 37 tested fatty acids, six were detected under our experimental conditions (Table 3). Palmitic (C16:0), oleic (C18:1n9c) and linoleic (C18:2n6c) acids were the major fatty acids, accounting for more than 90% of the total fatty acids, whereas stearic, linolenic and arachidic acids were found in lesser amounts. The major saturated fatty acid was palmitic acid, which varied from 12.8 to 13.7% with an average of 13.4%, showing no statistically significant difference among the eight tested varieties. The two major unsaturated fatty acids were oleic and linoleic acids, which ranged from 43.8 to 49.3% and from 34.7 to 40.3% with averages of 46.3 and 37.4%, respectively. Similar fatty acid compositions were found in Australian oats by Zhou et al. (1998), who reported 39.8% oleic and 37.5% linoleic acid content. The presence of high amounts of monounsaturated (oleic acid) and polyunsaturated fatty acids (linoleic and linolenic acids) in adlay suggested its health beneficial effects, as these fatty acids may decrease blood cholesterol levels (Hargrove et al., 2001) and improve HDL fluidity (Villa et al., 2002). Our results suggest that adlay seeds could be an alternative source of food oil based on the large quantities of monounsaturated and polyunsaturated fatty acids.

Correlationship among seed phytonutrient contents

Statistical correlationships among detected phytonutrients: 7 vitamin E isomers, squalene, 3 phytosterols and 6 fatty acids were tested. Squalene content exhibited highly positive correlationships with campesterol (r = 0.853**), stigmasterol (r = 0.873**) and β-sitosterol (r = 0.779**), while relatively low correlationship with vitamin E isomers were observed (Table 4). Such observations may result from the fact that phytosterols are produced by using the squalene as a preceeding material for their synthesis (Piironen et al., 2000), and consequently the correlationship between squalene and 3 tested phytosterols were higher compared to the relationship between squalene and tocopherols. Among the seven vitamin E isomers

Table 4. Correlationships among phytonutrients in adlay seeds: vitamin E isomers, squalene and phytosterols.

	α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	α-Tocotrienol	γ-Tocotrienol	δ-Tocotrienol	Squalene	Campesterol	Stigmasterol	β-sitosterol
α-Tocopherol	1	-0.141	0.038	-0.139	-0.398	0.161	-0.07	0.421*	0.461*	0.498*	0.601*
β-Tocopherol		1	0.226	0.285	0.250	0.006	0.212	0.004	0.232	-0.018	0.165
γ-Tocopherol			1	0.814*	0.791*	0.760**	0.536*	0.125	0.079	0.059	0.411*
δ-Tocopherol				1	0.661**	0.524**	0.537**	0.182	0.082	0.102	0.250
α-Tocotrienol					1	0.498*	0.37	-0.247	-0.246	-0.385	-0.038
γ-Tocotrienol						1	0.812**	0.317	0.215	0.292	0.556**
δ-Tocotrienol							1	0.297	0.223	0.329	0.426*
Squalene								1	0.853**	0.873**	0.779**
Campesterol									1	0.865**	0.871**
Stigmasterol										1	0.833**
β-sitosterol											1

*, **: Significant at $p < 0.05$ and $p < 0.01$, respectively.

analyzed, γ-tocopherol content showed higher positive correlationhips with other vitamin E isomers except for α- and β-tocopherol. In the case of 6 observed fatty acids, no correlationships could be observed with 7 vitamin E isomers, squalene and 3 phytosterols (data not shown), which may due to no close relationship in biosynthetic pathways between fatty acid and vitamin E nor phytosterols.

Conclusion

This study shows that adlay seeds are a rich source of phytonutrients, justifying the dietary food status of adlay in oriental countries. Among the eight tested vitamin E isomers, γ-tocopherol and γ-tocotrienol were the major forms of vitamin E, and β-sitosterol was the major phytosterol in all tested varieties. The major fatty acids were palmitic (C16:0), oleic (C18:1n9c) and linoleic (C18:2n6c) acids, and the presence of a higher composition of unsaturated fatty acids indicated the high potential of adlay seeds for promoting human health. Squalene content showed highly correlationships with phytosterols while it showed low correlationships with vitamin E isomers. Among the tested varieties, Yulmu 1 was the best variety in terms of nutraceutical compounds, with relatively high vitamin E, squalene and phytosterol contents. Thus, Yulmu 1 could be useful as a gene source for breeding a more nutrient-enriched variety of adlay.

REFERENCES

Awad AB, Fink CS (2000). Phytosterols as anticancer dietary components: Evidence and mechanism of action. J. Nutri. 130: 2127-2130.

Borsonelo EC, Galduroz JCF (2008). The role of polyunsaturated fatty acids (PUFAs) in development, aging and substance abuse disorders: Review and propositions. Prostaglandin, Leukotrienes Essent. Fatty Acids, 78: 237-245.

Chang HC, Huang YC, Hung WC (2003). Antiproliferative and chemo preventive effects of adlay seed on lung cancer in vitro and in vivo. J. Agric. Food Chem. 51: 3656-3660.

Choi Y, Lee J (2009). Antioxidant and antiproliferative properties of tocotrienol-rich fraction from grape seeds. Food Chem. 114: 1386-1390.

Ha DT, Trung TN, Tu NB, On TV, Nam NH, Men CV, Phuong TT, Bae KH (2010). Adlay seed extract (*Coix lachryma-jobi* L.) decreased adipocyte differentiation and increased glucose uptake in 3T3-L1 cells. J. Med. Food. 13(6): 1331-1339.

Hargrove RL, Etherton TD, Pearson TA, Harrison EH, Kris-Etherton PM (2001). Low-fat and high-monounsaturated fat diets decrease human low-density lipoprotein oxidative susceptibility in vitro. J. Nutri.131: 1758-1763.

Hidaka Y, Kaneda T, Amino N, Miyai K (1992). Chinese medicine, *Coix* seeds increase peripheral cytotoxic T and NK cells. Biotherapy, 5: 201-203.

Hsu HY, Lin BF, Lin JY, Kuo CC, Chiang W (2003). Suppression of allergic reactions by dehulled adlay in association with the balance of Th1/Th2 cell responses. J. Agric. Food Chem. 51: 3763-3769.

Huang DW, Chung CP, Kuo YH, Lin YL, Chiang W (2009). Identification of compounds in Adlay (Coix *lacryma-jobi* L. *var. ma-yuen* Stapf) seed hull extracts that inhibit lipopolysachharide- induced inflammation in RAW 264.7 macrophages. J. Agric. Food Chem. 57: 10651-10657.

Khor HT, Chieng DY (1997). Effect of squalene, tocotrienols and α- tocopherol supplementations in the diet on serum and liver lipids in hamsters. Nutri. Res. 17(3): 475-483.

Kim JK, Kim NH, Bang JK, Lee BK, Park CB, Lee BH (2000). Fatty acid composition analysis of major oil crops by one-step extraction/methylation method. Korean J. Crop Sci.

45(3): 211-215.
Kim SO, Yun SJ, Jung B, Lee EH, Hahm DY, Shim I, Lee HJ (2004). Hypolipidemic effects of crude extracts of adlay seed (*Coix lacryma jobi* var. *mayuen*) in obesity rat fed high fat diet: Relations of TNF-a and leptin mRNA expressions and serum lipid levels. Life Sci. 75: 1391-1404.
Lee MY, Lin HY, Cheng F, Chiang W, Kuo YH (2008). Isolation and characterization of new lactam compounds that inhibit lung and colon cancer cells from adlay (*Coix lacryma-jobi* L. *var. ma-yuen* Stapf) bran. Food Chem. Toxicol. 46: 1933-1939.
Lee YC, Chuah AM, Yamaguchi T, Takamura H, Matoba T (2008). Antioxidant Activity of Traditional Chinese Medicinal Herbs. Food Sci. Technol. Res. 14(2): 205-210.
Marangoni F, Poli A (2010). Phytosterol and cardiovascular health. Pharmacol. Res. 61(3): 193-199.
Mendilaharsu M, Stefani ED, Pellerini HD, Carzoglio J, Ronco A (1998). Phytosterols and risk of lung cancer: A case control study in Uruguay. Lung Cancer, 21: 37-45.
Moreda W, Perez-Camino MC, Cart A (2001). Gas and liquid chromatography of hydrocarbons in edible vegetable oils. J. Chromatogr. A, 936: 159-171.
Nagao T, Otsuka H, Kohda H, Sato T, Yamasaki K (1985). Benzoxazinones from *Coix lachrymal-jobi* L *var. ma-yuen.* Phytochemistry, 24(12): 2959-2962.
Nageshwari K, Banerjee R, Menon VP (1999). Effect of saturated, ω-3 and ω-6 polyunsaturated fatty acids on myocardial infarction. J. Nutr. Biochem. 10: 338-344.
Ohkatsu Y, Kajiyama T, Arai Y (2001). Antioxidant activities of tocopherols. Polym. Degrad. Stab. 72: 303-311.
Packer L (1995). Nutrition and biochemistry of the lipophilic antioxidants, vitamin E and carotenoids. In A.S.H. Ong, E. Niki, & L. Packer (Eds.), Nutrition, Lipids, Health and Disease (pp. 8-35). Champaign: AOCS Press.
Park KY, Kang CS, Lee YS, Lee YH, Lee YS (2004). Tocotrienol and tocopherol content in various plant seeds. Korean J. Crop Sci. 49(3): 207-210.
Piironen V, Lindsay DG, Miettinen TA, Toivo J, Lampi AM (2000). Plant sterols: biosynthesis, biological function and their importance to human nutrition. J. Sci. Food Agric. 80: 939-966.
Piironen V, Toivo J, Lampi AM (2002). Plant sterols in cereals and cereal products. Cereal Chem. 79(1): 148-154.
Ryan E, Galvin K, O'Connor TP, Maguire AR, O'Brien NM (2007). Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes. Plant Foods Hum. Nutri. 62: 85-91.
Shih CK, Chiang W, Kuo ML (2004). Effects of adlay on azoxymethane-induced colon carcinogenesis in rats. Food Chem. Toxicol. 42: 1339-1347.
Singh V, Moreau RA, Hicks KB (2003). Yield and phytosterol composition of oil extracted from grain sorghum and its wet-milled fractions. Cereal Chem. 80(2): 126-129.
Villa B, Calbresi L, Chiesa G, Rise P, Galli C, Sirtori C (2002). Omega-3 fatty acid ethyl esters increase heart rate variability in patients with coronary disease. Pharmacol. Res. 45(6): 475-478.
Wada S, Satomi Y, Murakoshi M, Noguchi N, Yoshikawa T, Nishino H (2005). Tumor suppressive effects of tocotrienol in vivo and in vitro. Cancer Lett, 229: 181-191.
Wang CY, Lin HT, Wu SC (2011). Influence of dietary supplementation with *Bacillus* fermented adlay on lipid metabolism, antioxidant status and intestinal microflora in hamsters. J. Sci. Food Agric. 91: 2271-2276.
Wu TT, Charles AL, Huang TC (2007). Determination of the contents of the main biological compounds of Adlay (*Coxi lachrymal-jobi*). Food Chem. 104: 1509-1515.
Yaqoob P (2002). Monounsaturated fatty acids and immune function. Eur. J. Clin. Nutri. 56: 9-13.
Yu F, Gao J, Zeng Y, Liu CX (2011). Effects of adlay seed oil on blood lipids and antioxidant capacity in hyperlipidemic rats. J. Sci. Food Agric. 91: 1843-1848.
Zhou MX, Holmes MG, Robards K, Helliwell S (1998). Fatty acid composition of lipids of Australian oats. J. Cereal Sci. 28: 311-319.