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Original Research Article

The Contents of Phytosterols, Squalene, and Vitamin E and the Composition of Fatty Acids of Korean Landrace *Setaria italica* and *Sorghum bicolar* Seeds

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Abstract - To characterize the nutraceutical property of Italian millet (*Setaria italica*) and sorghum (*Sorghum bicolor*), ten Korean landraces of each crop were collected and their vitamin E (tocopherols and tocotrienols), squalene and phytosterols (campesterol, stigmasterol and β-sitosterol) contents as well as fatty acid composition in seeds were evaluated. Italian millet seeds exhibited 5 forms of vitamin E isomers: three (α-, γ- and δ-) tocopherols and two (α- and γ-) tocotrienols, while sorghum seeds showed only three forms of vitamin E isomers: α- and γ-tocopherol and α-tocotrienol. In both crops, γ -tocopherol was the major constituent of vitamin E in terms of highest quantity. Total vitamin E content in Italian millet and sorghum landraces were 88.3 mg/kg and 44.3 mg/kg, respectively. Among three phytosterols (campesterol, stigmasterol and β-sitosterol) analyzed, β-sitosterol was the major form comprising about 85% and 65% in Italian millet and sorghum landraces, respectively. Total phytosterols content ranged from 443.0 to 568.5 mg/kg and 442.3 to 719.2 mg/kg in Italian millet and sorghum, respectively. Squalene, a precursor of phytosterols biosynthesis, ranged from 6.8 to 10.2 mg/kg in Italian millet and from 62.2 to 115.2 mg/kg in sorghum. Linoleic, oleic and palmitic acids were the major fatty acids in both of the crops and about 80% of the total fatty acids were unsaturated fatty acids. Among the tested landraces, M09 and S10 showed relatively higher proportion of phytonutrients, suggesting their potential as a gene source for further breeding program.

Key words - Phytonutrient, Cereal, Tocotrienol, Tocopherol, Campesterol, Sitosterol, Stigmasterol

Introduction

Italian millet (*Setaria italica*) and Sorghum (*Sorghum bicolor*) are world's sixth and fifth most important cereal grains, respectively, in terms of both production and cultivation area (FAO, 2011). They are inexpensive and nutritionally comparable or even superior to major cereals (Pathak *et al*., 2000; Duodu *et al*., 2003). These crops are staple foods that supply a major proportion of calories and protein to large segments of populations in the semi-arid tropical regions of Africa and Asia (O'Kennedy *et al*., 2006). The Italian millet and sorghum can also grow and give higher and more stable grain yields under poor soil and growing conditions.

In addition to starch, a major constituent of cereal crop seeds, the presence of various phytochemicals such as vitamins, polyphenols, flavonoids, minerals, phytosterols as well as fatty acids enrich the nutritional value of cereals. And consequently it is noteworthy to trace such health beneficial phytochemicals in seeds of cereal crops. Vitamin E, squalene, and phytosterols are health beneficial compounds present in the unsaponifiable lipid fraction of cereal crops. Vitamin E consisting of four tocopherols (α-, β-, γ-, and δ-tocopherol) and the corresponding tocotrienols (α-, β-, γ-, and δ-tocotrienol) is a fat soluble antioxidant and functions as scavengers of lipid peroxyl radicals. Tocopherol content in food is inversely associated with mortality from cardiovascular disease (Knekt *et al.*, 1994; Kushi *et al*., 1996). In addition, tocopherols, due to their capacity to quench free radical damage, play a putative role in prevention of Alzheimer's disease and cancer (Tucker and Townsend, 2005). Among the four tocopherol isomers, α-

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tocopherol is considered as the most biologically active form (Ohkatsu *et al*., 2001) and has the function of a radical-chain breaking antioxidant in membranes and lipoproteins, as well as in foods (Kamal-Eldin and Appelqvist, 1996). Tocotrienols, another form of vitamin E have been reported to exhibit health-beneficial effects similar to tocopherols; antioxidative, antiproliferative (Choi and Lee, 2009), anticancer (Wada *et al*., 2005), and cholesterol biosynthesis-inhibiting effects (Qureshi *et al*., 1995). Phytosterols, primarily β-sitosterol, campesterol, and stigmasterol are integral natural components of plant cell membranes that are abundant in vegetable oils, nuts, and grains (Weihrauch and Gardner, 1978). They have a variety of biological effects including serum-cholesterol lowering effects (Ostlund, 2004; Marangoni and Poli, 2010), anti-inflammatory, anti-oxidative, and anti-carcinogenic activities (de Jong *et al*., 2003). Several studies have shown that plant sterols inhibit the intestinal absorption of cholesterol, thereby lowering total plasma cholesterol and low-density lipoprotein (LDL) levels (de Jong *et al*., 2003). Squalene, a 30 carbon isoprenoid, is a key intermediate in cholesterol biosynthesis (Moreda *et al*., 2001) and is an important dietary cancer-chemopreventive agent (Smith, 2000). More recently, squalene has been shown to act as an antidote to reduce accidental drug-induced toxicities (Aguilera *et al*., 2005; Senthilkumar *et al*., 2006). The protective effect of squalene may be attributed to its ability to serve as an antioxidant; e.g., it has been demonstrated to be a potent quencher of singlet oxygen (Kohno *et al*., 1995) and protects against H_2O_2-induced sister chromatid exchange (SCE) in Chinese hamster V79 cells (O'Sullivan *et al*., 2002).

Most of phytonutrient studies on cereals, however, have been conducted intensively for major crops. There are several reports regarding the polyphenols, tannins and flavonoid content, antioxidant as well as antiradical activities and proximate nutrient composition in millet and sorghum (Awika and Rooney, 2004; Dykes and Rooney, 2006; Chethan and Malleshi, 2007). Information regarding lipophilic phytonutrients such as vitamin E, phytosterols, and fatty acid composition in these cereals is rare. Although Pirronen *et al.* (2002) and Ryan *et al.* (2007) have analyzed phytosterols in millet, only one variety had been selected and the variety name was not clearly identified. Singh *et al.* (2003) analyzed phytosterols in sorghum but the number of variety was only one. So, it will be noteworthy to analyze the lipophilic phytonutrients in various sorghum and millet landraces to select any landrace having higher content of phytonutrients for the breeding program. Hence, this study was mainly focused on evaluation of tocopherol, tocotrienol, squalene and phytosterol contents as well as fatty acid composition in seeds of Italian millet and sorghum landraces collected from Korea.

Materials and Methods

Sample collection

Grains of ten landraces of each sorghum and Italian millet were kindly donated from Sinlim Agricultural Cooperative Federation of Wonju City, Kangwon-Do, South Korea. The list of landraces and their Korean names are provided in Table 1. Grain samples delivered to analysis laboratory were ground to fine powders and stored at -80℃ prior to nutrient analyses conducted within 1 month after delivery.

Chemicals and Reagents

Authentic standards of squalene, campesterol, stigmasterol, and β-sitosterol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Standards of FAME (fatty acid methyl ester) were acquired from Supelco (Bellefonte, PA, USA), and vitamin E standards (tocopherols: α-, β-, γ-, and δ-tocopherol; and tocotrienols: α-, β-, γ- and δ-tocotrienol) were purchased from Merck (Darmstadt, Germany). Ascorbic acid, chloroform

Table 1. The name and abbreviations of Italian millet and sorghum landraces used in this experiment

Crop	Korean variety names and abbreviations of landraces
Italian millet	Hwangum Mejo (M01), Mejo (M02), Heuin Chajo (M03), Jangsoo Hwang Chajo (M04), Buksimie Chajo (M05), Hwang Chajo (M06), Auroon Chajo (M07), Ohl Jo (M08), Ggojang Jo (M09), Neut Jo (M10)
Sorghum	Chal Susu (S01), Ilbanchal Susu (S02), Bulgeunjangmok Susu (S03), Bulgeunjangsu Susu (S04), Me Susu (S05), Jangsu Susu (S06), Mongdang Susu (S07), Sikyung Susu (S08), Susongsaengie Susu (S09), Heun Susu (S10)

and anhydrous sodium sulfate were obtained from Samchun (Seoul, Republic of Korea), and benzene, ethanol, potassium hydroxide, sulfuric acid and n-heptane were purchased from Daejung (Seoul, Republic of Korea), and 2,2-dimethoxypropane was obtained from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals, including *n*-hexane (HPLC grade), *iso*-octane (2,2,4-trimethyl pentane; HPLC grade) and methanol (HPLC grade) were purchased from J.T. Baker (Phillipsburg, NJ, USA).

Vitamin E, squalene and phytosterols analysis

The samples for the analyses of vitamin E isomers (α-, β-, γ-, and δ-tocopherol and α-, β-, γ-, and δ-tocotrienol), phytosterols (campesterol, stigmasterol and β-sitosterol) and squalene were prepared and analyzed based upon the procedure previously described by Park *et al*. (2004) and Bhandari *et al*. (2012). Briefly, the powdered samples (1.0 g) were placed in a 50 mL tube, and 0.1 g ascorbic acid was added as an antioxidant along with 10 mL ethanol prior to shaking in a water bath at 80℃ for 10 min. Then, 300 μL of 44% KOH was added and the mixture was shaken for saponification for 18 min in a water bath at 80℃. The tubes were cooled rapidly in an ice bucket, after with 10 mL n-hexane and 10 mL of distilled water were added, mixed, and centrifuged for 10 min at 1000 rpm; and the upper hexane layer was collected. This process was repeated three times, and the collected hexane layers were pooled and washed three times with 10 mL distilled water and passed through anhydrous Na_2SO_4 to remove water, concentrated in a rotary evaporator and dissolved in *iso*-octane. Then the samples were analyzed by gas chromatography (Varian 3800, Palo Alto, CA, USA). The analysis was performed with a capillary column (CP-SIL 8CB, 30 × 0.25 mm, 0.4 μm film thickness) with the injector and FID temperatures set at 290℃. The injection volume was 1 μL with a split ratio of 1:20 and the carrier gas (He) flow rate was 1.0 mL/min. The oven temperature was initially set at 220℃ for 2 min, increased to 290℃ by 5℃/min, held for 14 min, and then increased to 310℃ at a rate of 10℃/min. Peak identifications were conducted based upon the retention times of authentic standard compounds.

Fatty acid composition analysis

Samples for fatty acid composition analysis were prepared according to Kim *et al*. (2000) with slight modifications. Powdered samples (0.2 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 hr at 80℃ in a water bath and cooled to room temperature. The heptane layer was collected by centrifugation and was injected into a gas chromatography (Varian, CP-3800, Palo Alto, CA, USA) equipped with a capillary column (CP-SIL 88 CB FAME, 50 × 0.25 mm, 0.2 μm film thickness; Supelco, Bellefonte, PA, USA). The temperatures for injector and FID detector were set at 210℃ and 290℃, respectively and the carrier gas was helium. The injection volume was 1 μL with a split ratio of 1:50 on constant column flow (1.0 mL/min). Oven temperature was initially set on 100℃ for 5 min, then raised up to 180℃ at 4℃ /min increasing rate, held for 5 minutes and then increased to 210℃ by 5℃/min, and held for 20 min. A mixture of 37 FAME standards was used to identify the peaks based upon the retention time. The relative percentage of each identified fatty acid was calculated based upon their peak area and used for the composition of each fatty acid.

Data analysis

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

Results and Discussion

Vitamin E content

Among 8 vitamin E isomer tested, 5 vitamin E isomers (3 tocopherols and 2 tocotrienols) could be quantified in Italian millet while only 3 vitamin E isomers (2 tocopherols and 1 tocotrienol) were observed in sorghum. In both of the crops, the major form of vitamin E isomer was γ-tocopherol which showed 63.1 mg/kg and 37.5 mg/kg in average of tested landraces corresponding to 71% and 85% of total vitamin E in Italian millet and sorghum, respectively (Table 2 and 3). In the case of Italian millet, total tocopherol content ranged from 66.3 mg/kg (M07) to 94.2 mg/kg (M01) with an average of 79.1 mg/kg. Our findings for the tocopherols were higher

Table 2. Tocopherol and tocotrienol contents in seeds of Italian millet landraces (mg/kg)

Landrace	α-tocopherol	γ-tocopherol	δ-tocopherol	α-tocotrienol	γ-tocotrienol	Total tocopherol	Total tocotrienol	Total vitamin E
M01	11.7[z]cd[y]	80.7a	1.8a	1.7a	7.8bcd	94.2a	9.5bcd	101.9a
M02	13.9bc	73.7a	2.0a	1.6ab	7.6bcde	89.5ab	9.3bcde	98.8ab
M03	17.5a	56.0bc	1.4a	1.7ab	9.2ab	75.0cd	10.7ab	85.6cd
M04	19.3a	56.8b	2.1a	1.3bcde	8.3bcd	78.1c	9.6bcd	87.7bc
M05	14.6b	62.0b	1.5a	1.4abcd	8.7bc	78.1c	10.1bc	88.2bc
M06	17.0a	62.8b	1.3a	1.1de	6.8def	81.0cd	7.9def	88.9bc
M07	17.3a	48.7c	0.3a	1.0e	7.4cdef	66.3d	8.4cdef	74.7d
M08	10.5d	64.2b	0.4a	1.5abcd	6.0f	75.0cd	7.5ef	82.6cd
M09	13.5bc	64.0b	0.5a	1.5abc	10.4a	77.9c	11.9a	89.9bc
M10	12.5bcd	62.6b	0.4a	1.2cde	6.2ef	75.5cd	7.4f	82.9cd
Average	14.8	63.1	1.2	1.4	7.8	79.1	9.2	88.3

[z]Values are mean of three independent replications.
[y]Means followed by different letters within a column are significantly different at $p < 0.05$ by Duncan's multiple range tests.

Table 3. Tocopherol and tocotrienol contents in seeds of sorghum landraces (mg/kg)

Landrace	α-tocopherol	γ-tocopherol	α-tocotrienol	Total tocopherol	Total tocotrienol	Total vitamin E
S01	4.5[z]ab[y]	43.0ab	2.1b	48.4ab	2.1b	50.6ab
S02	4.9ab	44.4a	1.8bc	50.6a	1.8ab	52.5a
S03	3.9b	41.7ab	1.5cd	46.9abc	1.5cd	48.3abc
S04	4.6ab	27.1d	1.3a	31.9a	1.3d	33.2e
S05	5.1a	33.5cd	1.5cd	38.7cde	1.5cd	40.2cde
S06	4.0ab	36.8bc	1.8bc	41.3bcd	1.8bc	43.1bcd
S07	4.3ab	32.5cd	3.6a	37.0de	3.6a	40.5cde
S08	4.1ab	31.1cd	1.7bcd	35.1de	1.7bcd	36.8de
S09	4.5ab	36.5bc	1.6cd	41.4bcd	1.6bcd	43.1bcd
S10	4.4ab	47.8a	1.6bcd	52.8a	1.6cd	54.4a
Average	4.4	37.5	1.8	42.4	1.8	44.3

[z]Values are mean of three independent replications.
[y]Means followed by different letters within a column are significantly different at $p < 0.05$ by Duncan's multiple range tests.

than those of Ryan *et al*. (2007), who reported 26 mg/kg of total tocopherol in Italian millet. Tocopherols content in this study were higher than in barley (16 mg/kg), buckwheat (46 mg/kg) and maize (13 mg/kg) (Ryan *et al*., 2007). We observed only two forms of tocotrienol isomers; α- and γ-tocotrienol in Italian millet landraces and the total tocotrienols content varied from 7.4 mg/kg (M10) to 11.9 mg/kg (M09) with an average of 9.2 mg/kg. Total vitamin E content in Italian millet seeds varied from 74.7 mg/kg in M07 landrace to 101.9 mg/kg in M01 landrace with an average of 88.3 mg/kg. Compared to Italian millet, tested sorghum landraces showed somewhat different vitamin E content pattern in that only 3 vitamin E isomers could be quantified under our experimental conditions. The γ-tocopherol, a dominant form of vitamin E, varied from 27.1 mg/kg in S04 to 47.8 mg/kg in S10 landrace (Table 3). Total vitamin E content which varied from 33.2 mg/kg (S04) to 54.4 mg/kg (S10) with an average of 44.3 mg/kg was lower than that in Italian millet. Except for δ-tocopherol in Italian millet, other vitamin E isomers showed statistically significant values among the landraces.

Table 4. Squalene and phytosterols contents in seeds of Italian millet landraces (mg/kg)

Landrace	Squalene	Campesterol	Stigmasterol	β-sitosterol	Total phytosterol
M01	7.9[z]bc[y]	32.2a	35.4bc	420.2bcd	487.9abc
M02	7.7bc	28.4a	32.9c	443.1abcd	504.4abc
M03	9.0abc	27.5a	45.1ab	427.3abcd	500.0abc
M04	9.4ba	31.2a	44.6ab	427.3abcd	503.1abc
M05	7.4ab	32.7a	45.8ab	415.7bcd	494.1abc
M06	7.2ab	27.8a	37.5abc	377.7d	443.0c
M07	7.9ab	27.3a	46.7a	403.2cd	477.2bc
M08	6.8c	25.3a	40.2abc	453.3abc	518.7abc
M09	10.2a	30.2a	43.1abc	495.3a	568.5a
M10	9.2ab	24.9a	40.4abc	482.4ab	547.7bc
Average	8.3	28.7	41.2	434.5	504.5

[z]Values are mean of three independent replications.
[y]Means followed by different letters within a column are significantly different at $p < 0.05$ by Duncan's multiple range tests.

Table 5. Squalene and phytosterols contents in seeds of sorghum landraces (mg/kg)

Landrace	Squalene	Campesterol	Stigmasterol	β-sitosterol	Total phytosterol
S01	78.1[z]bc[y]	67.6c	59.0f	315.7cd	442.3cd
S02	80.1bc	69.8c	56.8f	321.4cd	448.1cd
S03	84.0b	61.1cd	48.2f	295.9d	405.2d
S04	62.2d	49.4d	104.1cde	321.4cd	474.9c
S05	68.7d	54.9d	93.9e	307.5d	456.3cd
S06	86.4b	69.9c	99.1de	329.6cd	498.6c
S07	111.3a	102.6a	120.3bc	351.8bc	574.7b
S08	115.2a	92.4b	123.7b	375.2b	591.2b
S09	83.2b	87.6b	113.0bcd	368.1b	568.7b
S10	77.9bc	100.1a	147.1a	472.0a	719.2a
Average	84.7	75.5	96.5	345.9	517.9

[z]Values are mean of three independent replications.
[y]Means followed by different letters within a column are significantly different at $p < 0.05$ by Duncan's multiple range tests.

Phytosterols content

The levels of phytosterols (campesterol, stigmasterol and β-sitosterol) in Italian millet seeds were analyzed. β-sitosterol, which ranged from 377.7 mg/kg in M06 to 495.3 mg/kg in M09, was present in highest quantity comprising about 85% of total phytosterol content, which was followed by stigmasterol and then campesterol (Table 4). Total phytosterol content in Italian millet landraces varied from 443.0 (M06) to 568.5 mg/kg (M09) with the average of 504.5 mg/kg. Average quantity of β-sitosterol in this study was quite similar to the Ryan *et al*. (2007) and higher than reported by Piironen *et al*. (2002), however we found lower campesterol (28.7 mg/kg) and higher stigmasterol (41.2 mg/kg) that might be due to the genetic differences of Italian millet landraces. Average total phytosterols content of Italian millet was 504.5 mg/kg, which was higher than in maize (436 mg/kg) and similar to barley (504 mg/kg) (Ryan *et al*., 2007). Similar to the Italian millet, sorghum also possessed higher β-sitosterol compared to campesterol and stigmasterol (Table 5) and sorghum's average contents of campesterol (75.5 mg/kg) and stigmasterol (96.5 mg/kg) were higher than those in Italian millet. The phytosterols content in sorghum was similar to Singh *et al*. (2003) who reported 460

to 510 mg/kg of total phytosterols in grain sorghum and higher than in maize and barley (Ryan *et al*., 2007). Among tested ten sorghum landraces we found exceptionally higher total phytosterols content in S10 (719.2 mg/kg) suggesting its superiority than other landraces in terms of phytosterols content. All these results suggest that Italian millet and sorghum can be used as a good source of phytosterols for human diet compared to other cereals for the improvement of human health based upon phytosterols' lowering the serum-cholesterol (Marangoni and Poli, 2010) and anti-carcinogenic activities (de Jong *et al*., 2003).

Squalene content

Squalene, a precursor of biosynthetic pathway of phytosterols, content in ten Italian millet landraces varied from 6.8 mg/kg (M08) to 10.2 mg/kg (M09) (Table 4), and the average squalene content was 8.3 mg/kg. Compared to the Italian millet, sorghum landraces exhibited higher squalene content in that it varied from 62.2 mg/kg (S04) to 115.2 mg/kg (S08) with an average of 84.7 mg/kg (Table 5). In both crop cases, statistically significant difference could be observed among landraces. The present study showed relatively higher squalene content in sorghum landraces compared to that in barley (2.0 mg/kg), maize (16.0 mg/kg), spelt (20.0 mg/kg) and buckwheat (19.0 mg/kg) (Ryan *et al*., 2007) suggesting sorghum as a good source of squalene.

Fatty acid composition

Among thirty seven fatty acids screened, seven fatty acids could be quantified in Italian millet. Palmitic (12.3%), oleic (11.7%) and linoleic (65.2%) acids were the major fatty acids comprising over 85% of total fatty acids (Table 6). Other fatty acids were stearic, linolenic, arachidic and behenic acids, which consisted 5.6%, 3.4%, 1.4% and 0.5% of total fatty acids, respectively. The composition of saturated fatty acids (SFA) ranged from 18.3% to 21.8%, while mono-unsaturated (MUFA) and poly-unsaturated fatty acids (PUFA) were the major forms consisting almost 80% of total fatty acids in Italian millet seeds. Unlike the case of Italian millet, behenic acid could not be detected in sorghum seeds under our experimental conditions and six fatty acids were quantified. Fatty acids composition of sorghum seeds was quite similar to Italian millet. Sorghum also exhibited palmitic, oleic and linoleic acids as major fatty acids with an average composition of 15.5%, 34.9%, and 45.8% of total fatty acids, respectively, accounting more than 95% of total fatty acids (Table 7). Similar compositional ratio was also reported by Mehmood *et al*. (2008). However, sorghum seeds exhibited relatively higher

Table 6. Fatty acid composition in seeds of Italian millet landraces (%)

Landrace	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	Linolenic acid (C18:3)	Arachidic acid (C20:0)	Behenic acid (C22:0)	Saturated fatty acids	Mono-unsaturated fatty acid	Poly-unsaturated fatty acids
M01	12.19[z]ab[y]	6.05b	12.56a	64.50c	2.84e	1.40bc	0.46bc	20.10b	12.56a	67.34d
M02	12.13ab	6.08b	12.60a	64.34cd	2.99de	1.38bc	0.48bc	20.08b	12.60a	67.34d
M03	12.06ab	7.25a	11.96b	62.78e	3.46c	1.78a	0.70a	21.80a	11.96b	66.24e
M04	11.84ab	4.62e	11.19cd	67.02d	3.43c	1.32bcd	0.58abc	18.36c	11.19cd	70.45a
M05	13.11a	4.95d	10.33e	65.76b	3.71abc	1.49b	0.64ba	20.19b	10.33e	69.48ab
M06	11.42b	4.80de	11.45c	67.13a	3.16d	1.46b	0.58abc	18.26c	11.45c	70.29a
M07	11.80ab	4.57e	11.30c	66.91a	3.51bc	1.34bcd	0.58abc	18.28c	11.30c	70.42a
M08	11.67ab	6.16b	12.45a	65.07bc	2.98de	1.24bcd	0.43bc	19.49bc	12.45a	68.05cd
M09	13.15a	6.01b	10.77d	64.84bc	3.79ab	1.04d	0.41c	20.61ab	10.77d	68.62bc
M10	13.13a	5.37c	12.65a	63.39de	3.88a	1.14cd	0.44bc	20.08b	12.65a	67.27d
Average	12.25	5.58	11.73	65.17	3.38	1.36	0.53	19.73	11.73	68.55

[z]Values are mean of three independent replications.

[y]Means followed by different letters within a column are significantly different at $p < 0.05$ by Duncan's multiple range tests.

Table 7. Fatty acid composition in seeds of sorghum landraces (%)

Landrace	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	Linolenic acid (C18:3)	Arachidic acid (C20:0)	Saturated fatty acids	Mono-unsaturated fatty acid	Poly-unsaturated fatty acids
S01	15.22[z]cde[y]	1.82abc	35.71b	45.27cd	1.51de	0.32ab	17.36bc	35.71b	46.78cd
S02	14.90de	1.91bc	36.03b	44.82d	1.78abc	0.33ab	17.15bc	36.03b	46.62d
S03	14.63e	2.02a	35.11c	45.77bc	1.82ab	0.45a	17.10bc	35.11c	47.59b
S04	16.75a	1.69bcd	33.78d	45.80bc	1.69bcd	0.20b	18.91a	33.78d	47.08b
S05	15.82bd	1.84abc	29.52e	51.10a	1.41e	0.24b	17.90ab	29.52e	52.50a
S06	15.51bcd	1.86abc	34.86a	45.58bcd	1.79abc	0.33ab	17.70b	34.86c	47.37bc
S07	16.14b	1.60cd	37.77a	42.08e	1.99a	0.31ab	18.05ab	37.77a	44.07e
S08	15.39bcde	1.91ab	34.71c	46.20b	1.54de	0.27b	17.60bc	34.71c	47.49b
S09	15.59bcd	1.56d	35.97b	44.92d	1.58cde	0.27b	17.42bc	35.97b	46.50d
S10	14.62e	1.74bcd	35.72b	45.92bc	1.66bcd	0.27b	16.65c	35.72b	47.58b
Average	15.46	1.79	34.92	45.75	1.68	0.30	17.58	34.94	47.36

[z]Values are mean of three independent replications.
[y]Means followed by different letters within a column are significantly different at $p < 0.05$ by Duncan's multiple range tests.

Table 8. Correlationships among phytonutrients in Italian millet seeds

Phytonutrients	Stearic acid	Oleic acid	Linoleic acid	Arachidic acid	Linolenic acid	Behenic acid	Squalene	Campe-sterol	Stigma-sterol	β-sito-sterol	α-toco-pherol	α-toco-trienol	γ-toco-pherol	γ-toco-trienol	δ-toco-pherol
Palmitic acid	-0.02	-0.338	-0.491**	-0.313	0.552**	0.061	0.593**	0.332	0.237	0.444**	-0.185	0.269	0.056	0.378*	0.096
Stearic acid		0.475**	-0.766**	0.272	-0.238	-0.106	0.05	-0.129	-0.178	0.18	-0.339	0.636**	0.309	0.212	0.046
Oleic acid			-0.443*	0.024	-0.521**	-0.352	-0.247	-0.314	-0.524**	0.047	-0.449*	0.155	0.419*	-0.555**	-0.052
Linoleic acid				-0.137	-0.129	0.003	-0.353	0.004	0.065	-0.381*	0.366*	-0.543**	-0.29	-0.17	-0.075
Arachidic acid					-0.223	0.704**	-0.161	0.16	0.187	-0.272	0.433*	0.096	0.053	0.17	0.238
Linolenic acid						0.051	0.453**	-0.089	0.455*	0.172	0.228	-0.199	-0.498**	0.338	-0.069
Behenic acid							0.242	0.395**	0.383*	-0.068	0.560**	-0.169	-0.279	0.234	0.012
Squalene								0.399**	0.31	0.524**	0.247	0.142	-0.107	0.530**	0.003
Campesterol									0.282	0.582**	0.155	0.044	0.296	0.384*	0.104
Stigmasterol										0.239	0.394*	-0.198	-0.375	0.367*	-0.025
β-sitosterol											-0.235	0.124	0.262	0.223	-0.151
α-tocopherol												-0.181	-0.385	0.392*	0.294
α-tocotrienol													0.481	0.439*	0.546**
γ-tocopherol														0.111	0.291
γ-tocotrienol															0.264

*, ** Correlation is significant at $p < 0.05$ and $p < 0.01$ levels, respectively.

composition of oleic acid (34.9%) compared to Italian millet. The other fatty acids were stearic acid (1.8%), linolenic acid (1.7%) and arachidic acids (0.3%). In the present study, no special landrace was noticed having exceptionally higher composition of fatty acids than other. The saturated fatty acid of sorghum seeds ranged from 16.7% (S10) to 18.9% (S04) with an average of 17.6%. Similar to the Italian millet, sorghum seeds also exhibited about 80% of total unsaturated fatty acids. All tested sorghum landraces showed over 44.1% PUFA. High composition of unsaturated fatty acid in both Italian millet and sorghum seeds suggested higher health beneficial value of these crops since these unsaturated fatty acids may decrease the blood cholesterol levels (Hargrove *et al*., 2001), modulate immune function, and decrease susceptibility of

Table 9. Correlationships among phytonutrients in sorghum seeds

Phytonutrients	Stearic acid	Oleic acid	Linoleic acid	Arachidic acid	Linolenic acid	Squalene	Campe-sterol	Stigma-sterol	β-sito-sterol	α-toco-pherol	α-toco-trienol	γ-toco-pherol
Palmitic acid	-0.446*	-0.228	-0.07	-0.157	0.056	-0.171	-0.186	0.119	-0.29	-0.015	0.16	-0.760**
Stearic acid		-0.241	0.302	0.068	-0.073	-0.112	-0.44*	-0.501*	-0.317	-0.194	-0.279	0.163
Oleic acid			-0.942**	0.23	0.542**	0.402*	0.561**	0.079	0.268	-0.279	0.537**	0.285
Linoleic acid				-0.293	-0.668**	-0.352	-0.452	-0.037	-0.106	0.335	-0.592**	-0.056
Arachidic acid					0.425*	0.06	-0.111	-0.381*	-0.331	-0.364	0.045	0.238
Linoenic acid						0.29	0.181	-0.036	-0.017	-0.261	0.454*	0.016
Squalene							0.532**	0.333	0.239	-0.285	0.401*	-0.106
Campesterol								0.611**	0.776**	0.091	0.518**	0.300
Stigmasterol									0.727**	0.083	0.130	-0.126
β-sitosterol										0.252	0.083	0.406*
α-tocopherol											0.073	0.36
α-tocotrienol												0.041

*, ** Correlation is significant at $p < 0.05$ and $p < 0.01$ levels, respectively.

oxidation of LDL and improve the fluidity of HDL (Villa *et al*., 2002). These results suggest that sorghum and Italian millet seeds may serve as good sources of healthy food due to high ratio of unsaturated fatty acids.

Correlationship among phytonutrients

Statistical correlationships among phytonutrients (vitamin E, squalene, phytosterols and fatty acids) were analyzed for both Italian millet and sorghum seeds. In the case of Italian millet seeds, squalene showed positive correlationship with campesterol (r = 0.399**), β-sitosterol (r = 0.524**) and α-tocopherol (r = 0.530**) as well as with palmitic acid (r = 0.593**) and linolenic acid (r = 0.453**) (Table 8). Among the vitamin E isomers α-tocotrienol showed somewhat high positive correlationship with δ-tocopherol (r = 0.546**). Among phytosterols β-sitosterol showed positive correlationship with campesterol (r = 0.582**), but not with stigmasterol (r = 0.239^{NS}). Sorghum seeds exhibited somewhat different correlationship among phytonutrients compared to Italian millet in that squalene showed positive correlationship only with campesterol (r = 0.532**) and β-sitosterol exhibited correlationship with both campesterol (r = 0.776**) and stigmasterol (r = 0.727**) (Table 9). The higher positive correlationships among phytosterols and squalene observed in both Italian millet and sorghum may result from the facts that phytosterols are produced by using squalene as a preceding material for their synthesis (Piironen *et al*., 2000 and references there in).

Conclusion

This study shows that both Italian millet and sorghum are a rich source of phytonutrients, justifying the dietary food status of these crops in various countries. In both sorghum and Italian millet landraces, γ-tocopherol was present in highest quantity among vitamin E isomers and β-sitosterol was the major phytosterol. The presence of higher content of vitamin E and phytosterol in Italian millet and sorghum compared to other cereal crops may enrich their nutritional value as a cereal crop. The major fatty acids were linoleic (C18:2n6c), oleic (C18:1n9c) and palmitic (C16:0) acids. High composition of such unsaturated fatty acids suggested sorghum and Italian millet as healthy food source. Among tested ones, sorghum landrace S10 and Italian millet landrace M09 exhibited superiority in term of phytochemicals suggesting these two landraces as a good gene source for further breeding programs.

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Literature Cited

Aguilera, Y., M.E. Dorado, F.A. Prada, J.J. Martinez, A. Quesada and V. R. Gutierrez. 2005. The protective role of squalene in alcohol damage in the chick embryo retina. Exp. Eye Res. 80:535-543.

Awika, J.M. and L.W. Rooney. 2004. Sorghum phytochemicals and their potential impact on human health. Phytochemistry 65:1199-1221.

Bhandari, S.R., S. Basnet, K.H. Chung, K.H. Ryu and Y.S. Lee. 2012. Comparisons of nutritional and phytochemical property of genetically modified CMV-resistant red pepper and its parental cultivar. Hort. Environ. Biotechnol. 53:151-157.

Chethan, S. and N.G. Malleshi. 2007. Finger millet polyphenols: characterization and their nutraceutical potential. Am. J. Food Technol. 2:582-592.

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

de Jong, A., J. Plat and R.P. Mensink. 2003. Metabolic effects of plant sterols and stanols. J. Nutr. Biochem. 14:362-369.

Duodu, K.G., J.R.N. Taylor, P.S. Belton and B.R. Hamaker. 2003. Factors affecting sorghum protein digestibility. J. Cereal Sci. 38:117-131.

Dykes, L. and L.W. Rooney. 2006. Sorghum and millet phenols and antioxidants. J. Cereal Sci. 44:236-251.

FAO. 2011. FAOSTAT ProdStat database, yearly production. URL: <http://faostat.fao.org>.

Hargrove, R.L., T.D. Etherton, T.A. Pearson, E.H. Harrison and P.M. Kris-Etherton. 2001. Low-fat and high monounsaturated fat diets decrease human low density lipoprotein oxidative susceptibility *in vitro*. J. Nutr. 131:1758-1763.

Kamal-Eldin, A. and L.A. Appelqvist. 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31:671-701.

Kim, J.K., N.H. Kim, J.K. Bang, B.K. Lee, C.B. Park and B.H. Lee. 2000. Fatty acid composition analysis of major oil crops by one step extraction/methylation method. Korean J. Crop Sci. 45:211-215.

Knekt, P., A. Reunanen, R. Jarvinen, R. Seppanen, M. Heliovaara and A. Aromaa. 1994. Antioxidant vitamin intake and coronary mortality in a longitudinal population study. Am. J. Epidemiol. 139:1180-1189.

Kohno, Y., Y. Egawa, S. Itoh, S. Nagaoka, M. Takahashi and K. Mukai. 1995. Kinetic study of quenching reaction of singlet oxygen and scavenging reaction of free radicals by squalene in n-butanol. Biochem. Biophys. Acta 1256:52-56.

Kushi, L.H., A.R. Folsom, R.J. Prineas, P.J. Mink, Y. Wu and R.M. Bostick. 1996. Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. N. Engl. J. Med. 334:1156-1162.

Marangoni, F. and A. Poli. 2010. Phytosterols and cardiovascular health. Pharmacol. Res. 61:193-199.

Mehmood, S., I. Orhan, Z. Ahsan, S. Aslan and M. Gulfraz. 2008. Fatty acid composition of seed oil of different *Sorghum bicolor* varieties. Food Chem. 109:855-859.

Moreda, W., M.C. Perez-Camino and A. Cert. 2001. Gas and liquid chromatography of hydrocarbons in edible vegetable oils. J. Chromatogr. A 936:159-171.

Ohkatsu, Y., T. Kajiyama and Y. Arai. 2001. Antioxidant activities of tocopherols. Polym. Degrad. Stab. 72:303-311.

O'Kennedy, M.M., A. Grootboom and P.R. Shewry. 2006. Harnessing sorghum and millet biotechnology for food and health. J. Cereal Sci. 44:224-235.

Ostlund, R.E. Jr. 2004. Phytosterols and cholesterol metabolism. Curr. Opin. Lipidol. 15:37-41.

O'Sullivan, L., J.A. Woods and N.M. O'Brien. 2002. Squalene but not n-3 fatty acids protect against hydrogen peroxide-induced sister chromatid exchanges in Chinese hamster V79 cells. Nutr. Res. 22:847-857.

Park, K.Y., C.S. Kang, Y.S. Lee, Y.H. Lee and Y.S. Lee. 2004. Tocotrienol and tocopherol content in various plant seeds. Korean J. Crop Sci. 49:207-210.

Pathak, P., S. Srivastava and S. Grover. 2000. Development of food products based on millet, legumes and fenugreek seeds and their suitability in the diabetic diet. Int. J. Food Sci. Nutr. 51:409-414.

Piironen, V., D.G. Lindsay, T.A. Miettinen, J. Toivo and A.M. Lampi. 2000. Plant sterols: biosynthesis, biological function and their importance to human nutrition. J. Sci. Food Agric. 80:939-966.

Piironen, V., J. Toivo and A.M. Lampi. 2002. Plant sterols in cereals and cereal products. Cereal Chem. 79:148-154.

Qureshi, A.A., B.A. Bradlow, L. Brace, J. Manganello, D.M. Peterson, B.C. Pearce, J.J. Wright, A. Gapor and C.E. Elson. 1995. Response of hypercholesterolemic subjects to administration of tocotrienols. Lipids 30:1171-1177.

Ryan, E., K. Galvin, T.P. O'Connor, A.R. Maguire and N.M. O'Brien. 2007. Phytosterol, squalene, tocopherol content

and fatty acid profile of selected seeds, grains, and legumes. Plant Foods Hum. Nutr. 62:85-91.

Senthilkumar, S., T. Devaki, B.M. Manohar and M.S. Babu. 2006. Effect of squalene on cyclophosphamide-induced toxicity. Clin. Chim. Acta 364:335-342.

Singh, V., R.A. Moreau and K.B. Hicks. 2003. Yield and phytosterol composition of oil extracted from grain sorghum and its wet-milled fractions. Cereal Chem. 80:126-129.

Smith, T.J. 2000. Squalene: potential chemopreventive agent. Expert. Opin. Investig. Drugs 9:1841-1848.

Tucker, J.M. and D.M. Townsend. 2005. Alpha-tocopherol: roles in prevention and therapy of human disease. Biomed. Pharmacother. 59:380-387.

Villa, B., L. Calabresi, G. Chiesa, P. Rise, C. Galli and C.R. Sirtori. 2002. Omega-3 fatty acid ethyl esters increase heart rate variability in patients with coronary disease. Pharmacol. Res. 45:475-478.

Wada, S., Y. Satomi, M. Murakoshi, N. Noguchi, T. Yoshikawa and H. Nishino. 2005. Tumor suppressive effects of tocotrienol *in vivo* and *in vitro*. Cancer Lett. 229:181-191.

Weihrauch, J.L. and J.M. Gardner. 1978. Sterol content of foods of plant origin. J. Am. Diet. Assoc. 73:39-47.

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