Food Research International 51 (2013) 971–976

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Effect of genotype and cultivation location on β-sitosterol and α-, β-, γ-, and δ-tocopherols in sorghum

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ARTICLE INFO

Article history:
Received 26 November 2012
Accepted 16 February 2013

Keywords:
Sorghum
β-Sitosterol
Tocopherols
Genotype
Cultivation location

ABSTRACT

This study aims to examine variations in β-sitosterol and α-, β-, γ-, and δ-tocopherol composition and content in sorghum grains with different genotypes and cultivation areas (Wonju and Miryang in Korea). β-Sitosterol content was found to be higher in Wonju sorghum; however, total tocopherol content was found to be higher in Miryang sorghum. Moreover, the β-sitosterol content did not correlate with physical characteristics, including 100-seed weight and color values (*L*, *a*, and *b*), but it was negatively correlated with total tocopherol content. β-Tocopherol was a major tocopherol in sorghum, constituting approximately 40%–46% of the total tocopherol content, and it was highly correlated with the total tocopherol content. Furthermore, the α-tocopherol content also increased with increasing γ-tocopherol content. Among the 4 tocopherols, only δ-tocopherol content correlated with physical characteristics of sorghum grain. This study deepened our knowledge of the variations in β-sitosterol and α-, β-, γ-, and δ-tocopherol content in sorghum with respect to genotype and cultivation location. In addition, this study demonstrated a correlation between some physical characteristics of sorghum and β-sitosterol/tocopherol content, and this information can be useful for breeding programs that develop or breed sorghum varieties or manufacture sorghum-based foods containing high amounts of β-sitosterol and tocopherols.

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) originated in Africa and is usually cultivated in tropical, subtropical, and arid regions (Waniska & Rooney, 2000). In general, sorghum cultivation is robust under poor environmental conditions such as developed land, barren soil, and drought; therefore, the interest in sorghum as a food source is increasing in many countries. Moreover, because sorghum is a major source of calories and protein (Dicko, Gruppen, Traore, Voragen, & van Berkel, 2006), it is a staple cereal food in many African and Asian countries (Rooney & Waniska, 2000).

The *Sorghum* genus includes many species and subspecies such as grain sorghum, grass sorghum, and sweet sorghum, or broom sorghum, which are named according to their utility (Mehmood, Orhan, Ahsan, Aslan, & Gulfraz, 2008). Sorghum is used for food, animal feed, fibers, and bio-ethanol production; however, it is most typically used for animal feed in many countries. According to the Food and Agricultural Organization of the United Nations, world sorghum production was approximately 57 million tons in 2005, which ranked fifth after maize, rice, wheat, and barley (Dlamini, Taylor, & Rooney, 2007).

Sorghum contains levels of starch and other major nutrients similar to those of other cereals (Serna-Saldivar & Rooney, 1995). Furthermore, various bioactive compounds such as phenolic acids, anthocyanins, flavonoids, condensed tannins, phytosterols, and policosanols are found in sorghum (Awika & Rooney, 2004; Rooney & Waniska, 2000). Ferulic acid, *p*-coumaric acid, and vanillic acid found in the bran layer of the sorghum grain are the most abundant phenolic acids (Rooney & Waniska, 2000). Production of these bioactive compounds influences color, pericarp thickness, and growth, all of which are affected by genetic and environmental conditions (Dykes & Rooney, 2006; Svensson, Sekwati-Monang, Lutz, Schieber, & Gänzle, 2010). Sorghum, however, is a poor source of vitamins A and C, which are required to facilitate the absorption of zinc and iron (Michaelsen & Friis, 1998).

Because the sorghum grain contains various functional compounds, this food also provides several health and pharmaceutical benefits such as low digestibility; cholesterol reduction; and antioxidant, anti-inflammatory, antifungal, antibacterial, and anticarcinogenic activities (Dykes, Seitz, Rooney, & Rooney, 2009; Rooney & Awika, 2005; Rooney & Waniska, 2000). The antioxidant activity of bran in red sorghum is greater than that of blueberries, which are known to be a strong natural antioxidant food; in addition, this activity is 3–5 times higher than that of whole sorghum grain (Awika & Rooney, 2004). The antioxidant activity of sorghum is due to the production of phenolic compounds. Sorghum is also useful for patients with celiac disease because it is a gluten-free cereal (Taylor, Schober, & Bean, 2006).

Many studies on sorghum have described sorghum phenolic or flavonoid content and their functional health benefits, but these studies have not addressed the presence of other bioactive compounds such

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http://dx.doi.org/10.1016/j.foodres.2013.02.027

as phytosterols or vitamins. Thus, studies on phytosterols or vitamins in sorghum are very limited compared to other functional foods such as fruits and vegetables. Therefore, in this study, we focused on phytosterol (β-sitosterol) and vitamin E (α-, β-, γ-, and δ-tocopherols) because these compounds are well-known bioactive compounds with antioxidant, antifungal, and anticancer activities (Awad, Chan, Downie, & Fink, 2000; Bouic & Lamprecht, 1999; Munné-Bosch & Alegre, 2002; Pinheiro-Sant'Ana et al., 2011). In addition, we also investigated the effects of cultivation location on β-sitosterol and tocopherol content in sorghum grain. This study provides basic information on the composition and variety of bioactive compounds in sorghum based on different genotypes and environments.

2. Materials and methods

2.1. Chemicals and reagents

β-Sitosterol and α-, β-, γ-, and δ-tocopherols were purchased from Sigma Aldrich (St. Louis, MO). Ascorbic acid (L(+)-ascorbic acid) and potassium hydroxide (KOH) were from Samchun Chemicals (Gyeonggi-do, Korea). Sodium sulfate (Na_2SO_4) was from Yakuri Chemicals (Kyoto, Japan). Ethyl acetate, methanol, ethanol, hexane, isooctane, and distilled water were purchased from J. T. Baker (USA); all chemicals used were of HPLC grade.

2.2. Sorghum source and cultivation information

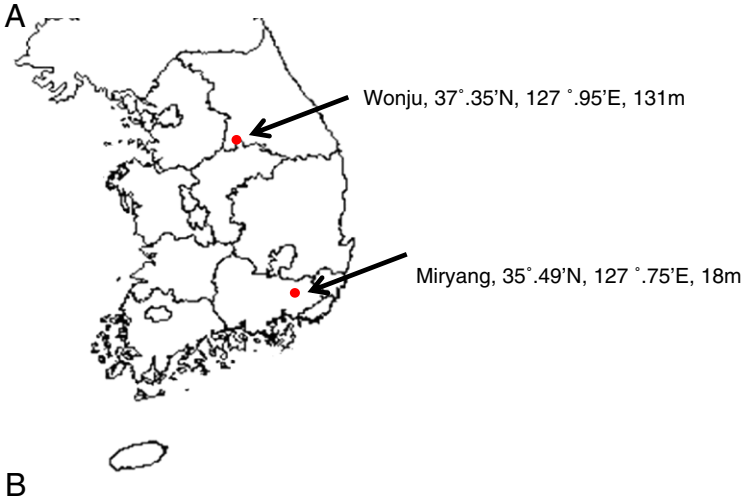
In 2010, 5 sorghum varieties were obtained from the Korea Rural Development Administration (RDA). Thereafter, each sorghum variety was cultivated at 2 different locations: Miryang (35°49′, 127°75′, 18 m) and Wonju (37°35′, 127°95′, 131 m) (Fig. 1A). Management, including pest control and fertilizer application during the cultivation period, was performed identically at both sites. Climate information (mean temperature, mean precipitation, etc.) of Wonju and Miryang in 2010 are also shown in Fig. 1B. Physical characteristics, including 100-seed weight and color of the 5 sorghum varieties are also summarized in the present study (refer to Table 3). Sorghum samples collected from 2 cultivation locations were stored in a dry keeper prior to analysis.

2.3. HPLC analysis of β-sitosterol

β-Sitosterol in sorghum grain was extracted as previously described with some modifications (Hung & Yen, 2001). Five grams of sorghum powder was soaked and mixed with 100 mL ethyl acetate for 24 h at room temperature and then filtered through Whatman no. 42 filter paper. After filtration, the sample was dried by a vacuum evaporator at 45 °C–50 °C and then lyophilized at below −30 °C. The residue was reconstituted with 2 mL ethyl acetate. Finally, the sample was filtered through a 0.45-μm pore size filter paper (TITAN syringe filter with a nylon membrane; Sun Sri, USA) and transferred to a 2-mL vial for high-performance liquid chromatography (HPLC).

An Acme 9000A Vitamin Analyzer HPLC (Young Lin Instrument Co., Ltd., Korea) was used for β-sitosterol analysis as per a modified method (Delgado-Zamarreno, Bustamante-Rangel, Martinez-Pelarda, & Carabias-Martinez, 2009). β-Sitosterol was separated with a YMC-Pack ODS AM-303 analytical column (5 μm, 250 nm × 4.6 mm ID), and absorbance was measured at 210 nm. The mobile phase consisted of methanol and distilled water (MeOH:H_2O = 100:2 v/v). A 20-μL sample aliquot was injected at a flow rate of 1 mL/min. The total isocratic HPLC run time was 60 min.

A



B

	Location		Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
Temperature, ˚C	Wonju	Max.	0.4	6.3	9.3	15.9	24.0	29.5	30.5	31.3	26.5	19.8	11.9	3.7
		Mean	-5.0	1.2	4.6	10.0	17.8	23.4	26.1	27.1	21.1	13.7	5.8	-1.6
		Min.	-10.1	-3.2	0.4	4.1	11.7	17.9	22.7	24.1	17.0	8.9	0.5	-6.4
	Miryang	Max.	6.7	9.4	11.8	17.1	24.8	30.0	30.7	33.2	29.2	23.0	15.8	8.4
		Mean	-0.4	3.6	6.9	10.8	18.0	23.6	26	28.1	23.0	15.8	6.8	1.4
		Min.	-6.5	-1.6	1.9	4.7	11.7	18.2	22.5	24.4	18.1	9.7	-0.5	-4.9
Duration of sunshine, h	Wonju		150.6	127.8	120.5	155.1	189.8	173.8	93.2	93.9	138.1	142.0	172.5	153.1
	Miryang		210.9	138.0	120.1	164.0	206.2	189.9	118.6	172.3	167.9	181.6	220.3	185
Precipitation, mm	Wonju		40.8	62.1	78.2	58.5	78.0	86.6	174.8	269.9	550.2	24.7	19.5	18.9
	Miryang		27.0	102.2	66.3	111.1	123.6	47.0	354.2	180.4	177.2	35.8	7.1	19.6
Mean wind speed, m/s	Wonju		1.1	1.1	1.6	1.7	1.4	1.1	1.1	1.1	0.9	0.8	1.1	1.4
	Miryang		1.4	1.7	2.1	2.0	1.9	1.6	1.6	1.7	1.6	1.3	1.4	1.7
Relative humid, %	Wonju		66.4	62.0	62.4	51.0	57.4	62.7	73.7	77.9	75.7	69.2	53.5	59.0
	Miryang		52.0	60.5	61.1	58.8	62.5	63.4	77.2	77.0	73.1	68.6	59.6	55.5

Fig. 1. Geographical (A) and climate information (B) of 2 cultivation locations (Wonju and Miryang) in 2010.