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Characterization of Total and Individual Sterols in Canola Sprouts

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Abstract In this study, the contents of total and individual phytosterols in sprouts made from seeds of seven canola (*Brassica napus* L.) lines (Acropolis, Banjo, Jetton, KS-7740, KSM3-1-124, Mussette and Virginia), grown at three locations in Virginia (Orange, Petersburg and Suffolk), were determined. Canola sprouts contained, on an average, 36.3 g sterols in 100 g of unsaponifiable matter (UNSAP), 10.7 mg sterols in 1 g of oil and 2.4 mg sterols in 1 g of dry sprouts. The contents of individual phytosterols (μg per g of oil) in canola sprouts were 1,162 brassicasterol, 3,799 campesterol, 34 stigmasterol, 5,359 β-sitosterol, 201 Δ^5-avenasterol and 97 Δ^7-stigmastenol. Canola lines had significant effects on the contents of oil, brassicasterol and campesterol. Locations had significant effects on the oil, UNSAP, total sterols, brassicasterol, stigmasterol and β-sitosterol. The oil content in canola sprouts was positively correlated with total sterols and Δ^5-avenasterol, whereas oil content was negatively correlated with brassicasterol content. In general, the contents of campesterol and β-sitosterol increased with an increase in total sterol content. The concentrations of sterols were in the following decreasing order: β-sitosterol > campesterol > brassicasterol > Δ^5-avenasterol > Δ^7-stigmastenol > stigmasterol. These results indicate that canola sprouts may have the potential as a natural source of dietary sterols and might be desirable for human nutrition.

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Introduction

Sprouts have long been considered a nutritious and healthy food. Historically, the Chinese have, consumed sprouts as a healthy food, especially for healing and rejuvenation [1]. In recent years, sprouts have become a popular healthy food in the USA and western meals. Numerous studies have demonstrated that sprouts are one of the most complete and nutritious foods [2, 3]. They are considered a predigested food, with a higher biological efficiency value and lower level of antiphysiological factors than raw or cooked seeds. Sprouts have been observed to contribute extensively to the immune system, as an excellent detoxificants [4]. Sprouts from seeds of cruciferous plants, such as Brussels sprouts, broccoli and cauliflower, contain substantial quantities of the sulforaphane (4-methylsulfinylbutyl isothiocyanate), which are potent inducers of detoxicating enzymes and act as strong cancer chemopreventive phytochemicals [4, 5].

Little is known about the sterol characteristics of sprouts, even though plant sterols or phytosterols are essential components of the membrane lipid bilayer, which are produced only by plants. They are isoprenoid-derived molecules that have essential functions in eukaryotes in general but especially in higher plants. Sterols with phospholipids play a functional role in the regulation of membrane fluidity and permeability [6]. The biochemical process of sterol acylation is believed to play a role in maintaining the free sterol content of cell membranes at their physiological levels [6].

Sterol biosynthesis and composition during germination of seeds have been extensively examined and reviewed [7, 8]. Phytosterols are present in three forms: free sterols, steryl esters and steryl glucosides, of which the free sterols comprise the major constituent of sterols fraction. Further, sterols in edible oil are typically present as free sterols and steryl ester of fatty acids [8]. In general, the accumulation of total sterol increases in membrane and organelle production and decreases in steryl glycosides with the utilization of the stored sterols during germination. In addition, β-sitosterol has been shown to be the major sterol in all fractions. Stigmasterol and campesterol have showed the greatest changes upon germination [7].

Plant sterols are known to promote human health. They reduce blood pressure, serum cholesterol level and risk of chronic heart disease [9–12]. The effectiveness of phytosterols in lowering the serum cholesterol level in humans has been extensively investigated [9–12]. These studies have demonstrated that phytosterol and phytostanol fatty acid esters have serum cholesterol-lowering properties and inhibit cholesterol absorption in the human small intestine. Further, plant sterols also have anti-inflammatory, antioxidant and anti-carcinogenic effects [13]. Recent clinical and nutritional studies have demonstrated that plant sterols and stanol esters lowered LDL cholesterol by 9–14% [10, 11]. The highest reduction in LDL cholesterol is achieved at 1.6–2.0 g/day therapeutic doses of plant sterol or stanol ester. Additionally, these studies led to the recommendations that consumption of phytosterols enriched food products or as functional foods such as margarine, milk, yoghurt, with plant sterols as bioactive components will reduce absorption of dietary and endogenous cholesterol by about 50%. In addition, phytostanols, especially sitostanol and campestanol, the hydrogenated derivatives of β-sitosterol and campesterol, respectively, are more effective in lowering serum cholesterol level than phytosterols [10–12].

Canola production is increasing worldwide and there is potential for a substantial market for canola sprouts. There is a total lack of information about sterols composition in canola sprouts therefore, the objectives of the present investigation were to characterize sterols in canola sprouts, to determine the effects canola line and growing location have on sterols traits of canola sprouts and to assess the potential of canola sprouts as a dietary source of therapeutic plant sterols.

Experimental Procedures

Reagent and Chemicals

All reagents were HPLC grade or higher and were obtained from Sigma–Aldrich Corporation (St. Louis, MO, USA) unless otherwise stated.

Plant Materials

Seeds of seven canola lines (Acropolis, Banjo, Jetton, KS-7740, KSM3-1-124, Mussette and Virginia) produced in Virginia at three locations (Orange, Petersburg and Suffolk) during the 2001–2002 crop season were used for this study. Sprout production and chemical analysis were performed in triplicate for each line from each location.

Sprout Production

Canola seeds (20 g), were sprouted for 6 days in a wide-mouthed jar (9 × 9 × 16 cm) covered with a mesh screen top to ensure sufficient air ventilation under laboratory conditions (22 °C temperature and 98% relative humidity and room lighting, 375l× for 10 h), as previously described [3]. At the end of sprouting, the fresh weights were recorded and expressed as fresh yield in grams. The water contents of the sprouts were determined after drying at 65 °C until constant weight and expressed as a percentage of moisture content.

Oil Content

The oil was extracted from 5 g ground, dried canola sprouts, at room temperature by homogenization with hexane/isopropanol (3:2, v/v) as described by Hamama et al. [14]. The oil was extracted three times from each sample and bulked to ensure full oil recovery. The oil content was determined gravimetrically after drying under vacuum at 40 °C and stored under nitrogen at −10 °C until analysis. The oil content was expressed as g/100 g dried sprouts.

Unsaponifiable Matter (UNSAP)

Oil samples (0.2 g) from canola sprouts were mixed with 50 μg cholesterol internal standard and saponified under nitrogen with 20% (w/v) of methanolic KOH overnight at room temperature as previously described [14]. The UNSAP extract was dried under N_2, determined gravimetrically and expressed as a percentage (w/w) of canola oil and dried sprouts.

Sterols

Phytosterols in the UNSAP were isolated and determined using the same methods and gas chromatography techniques as that described in our previous work [14–16]. Sterol fractions were silylated by 1 mL *N*,*O*-bis (silyltrimethyl) trifluoroacetamide in 1% trimethylchlorosilane in glass vials having Teflon-lined caps at 80 °C for 60 min and then analyzed using the same GC conditions as described by Hamama et al. [14]. The peaks were tentatively identified by

comparison of retention times (RT) with trimethylsilane (Me3Si) derivatives of standard sterols prepared under the same conditions and with relative retention times (RRT) reported in the literature [14–17]. Cholesterol was used as the reference sterol and for determination of a response factor (14). Total sterol concentrations were expressed as g/100 g of UNSAP and mg/g of oil and mg/g of dry sprouts. Individual sterols were expressed as μg/g of oil. The contents of oil, UNSAP, total sterols and individual sterols were determined in ungerminated seeds adopting the same techniques used for canola sprouts [14].

Statistical Analysis

All data were analyzed by analysis of variance procedures (PROC GLM) in version 6.11 of SAS [18]. Fisher's protected least significant difference (LSD) test was used for mean separation with a significance level of 5%.

Results and Discussion

Canola sprouts contained, on an average, 27.21 g oil in 100 g dry sprouts, 2.99 g UNSAP in 100 g of oil, 8.03 mg UNSAP in 1 g of dried canola sprouts, 36.3 g sterols in 100 g of UNSAP, 10.7 mg sterols in 1 g of oil and 2.4 mg sterols in 1 g of dry sprouts (Tables 1, 2). The lipid content of the sprouts was 5.4% on a fresh weight basis. The average contents of individual phytosterols (μg per g of oil) in canola sprouts were 1,162 brassicasterol, 3,799 campesterol, 34 stigmasterol, 5,359 β-sitosterol, 201 Δ^5-avenasterol and 97 Δ^7-stigmastenol (Tables 1, 2 and 3). In comparison, canola (*Brassica napus* L.) phytosterols comprise about 0.9% of seed oil (Table 3). The average contents of the sterols (μg per g of oil) in ungerminated seeds were 1,070 brassicasterol, 2,989 campesterol, 40 stigmasterol, 4,469 β-sitosterol, 198 Δ^5-avenasterol and 67 Δ^7-stigmastenol. The present results agree well with the Codex standard of desmethylsterols in canola and low erucic acid rapeseed oils [19, 20].

The concentrations of sterols (4-desmethyl sterols) in canola sprouts were in the following decreasing order: β-sitosterol > campesterol > brassicasterol > Δ^5-avenasterol > Δ^7-stigmastenol > stigmasterol. The data also demonstrated that canola lines had significant effects on contents of oil, brassicasterol and campesterol (Table 1). Canola line effects on UNSAP in the sprouts were not significant (Table 1). The minimal and maximal amounts of brassicasterol (881.7 and 1,513.2 μg/g oil) were observed in the Acropolis and Jetton lines, whereas minimal and maximal content of campesterol (3,171.7 and 4,078.5 μg/100 g oil) were observed in sprouts of the Virginia and Mussette lines, respectively. Of the seven canola lines, Acropolis and Virginia sprouts had the highest (29.1%) and lowest (25.4%) contents of oil, respectively.

Locations had significant effects on oil, UNSAP, total sterols, brassicasterol, stigmasterol and β-sitosterol content (Table 2). Location effects on UNSAP in canola sprouts were not significant. The effect of location on total sterol was significant when expressed as mg/g of oil or mg/g of

Table 1 Sterols in sprouts of seven canola cultivars grown at three locations during the 2001–2002 season in Virginia

Genotype	Moisture (%)	Oil (%)	UNSAP1[a]	UNSAP2[a]	S1[a]	S2[a]	S3[a]
Acropolis	85.52	29.05	2.90	8.40	35.53	10.33	2.47
Banjo	86.81	27.03	3.20	8.57	33.43	10.52	2.75
Jetton	86.74	26.33	3.15	8.15	37.57	11.62	2.57
KS7740	87.03	27.92	3.02	8.27	34.22	10.13	2.50
KSM3-1-124	85.65	26.85	2.87	7.62	37.77	10.53	2.28
Musette	86.08	27.88	2.93	8.07	38.48	11.00	2.42
Virginia	87.84	25.40	2.85	7.17	37.32	10.50	2.07
Mean	86.52	27.21	2.99	8.03	36.33	10.66	2.44
SD	0.77	1.55	0.39	1.04	4.74	1.39	0.59
Genotype	BS[b]	CS[b]	Ss[b]	βS[b]	Δ^5-A[b]		Δ^7-S[b]
Acropolis	881.67	3,911.5	26.33	5,227.0	187.00		84.83
Banjo	954.67	3,925.5	40.00	5,361.2	166.50		73.00
Jetton	1,513.2	3,617.7	46.00	6,120.3	213.50		95.83
KS7740	932.00	3,889.0	24.67	4,982.5	199.33		101.67
KSM3-1-124	1,346.0	4,002.2	34.83	4,805.5	243.33		85.8
Musette	1,024.5	4,078.5	28.50	5,522.3	212.17		132.17
Virginia	1,481.5	3,171.7	34.00	5,497.0	185.50		104.50
Mean	1,161.9	3,799.4	33.48	5,359.4	201.05		96.83
SD	225.7	438.0	14.78	740.9	54.60		39.70

[a] UNSAP1: (g/100 g oil), UNSAP2: (mg/g dry sprouts); *S1* total sterols g/100 g UNSAP, *S2* total sterols mg/g of oil, *S3* mg/g dry sprouts

[b] *BS* brassicasterol, *CS* campesterol, *SS* stigmasterol, *βS* β-sitosterol Δ^5-A Δ^5-avenasterol; Δ^7-S Δ^7-stigmastenol. All sterols expressed as μg/g of oil

Table 2 Sterols in sprouts of seven canola cultivars grown at three locations during the 2001–2002 season in Virginia

Location	Moisture (%)	Oil (%)	UNSAP1[a]	UNSAP2[a]	S1[a]	S2[a]	S3[a]
Orange	86.80	25.30	3.01	7.57	34.79	10.33	2.31
Petersburg	87.49	26.09	3.28	8.54	35.41	11.43	2.85
Suffolk	85.28	30.24	2.67	7.99	38.79	10.23	2.15
Mean	80.30	27.21	2.99	8.03	36.33	10.66	2.44
SD	0.77	1.55	0.39	1.04	4.74	1.39	0.59

Location	BS[b]	CS[b]	Ss[b]	βS[b]	Δ^5-A[b]	Δ^7-S[b]
Orange	1,212.3	3,632.6	39.50	5,128.1	196.50	117.36
Petersburg	1,250.1	4,040.2	25.07	5,840.6	184.64	78.64
Suffolk	1,023.4	3,725.4	35.86	5,109.6	222.00	94.50
Mean	1,161.9	3,799.4	33.48	5,359.4	201.05	96.83
SD	225.7	438.0	14.78	740.9	54.60	39.70

[a] UNSAP1: (g/100 g oil), UNSAP2: (mg/g dry sprouts), *S1* total sterols g/100 g UNSAP, *S2* total sterols mg/g of oil, *S3* mg/g dry sprouts

[b] *BS* brassicasterol, *CS* campesterol, *SS* stigmasterol *βS* *β*-sitosterol

Δ^5-A = Δ^5-avenasterol; Δ^7-S = Δ^7-stigmastenol. All sterols expressed as μg/g of oil

Table 3 Changes in contents of oil, total and individual sterols in sprouts of seven canola cultivars grown at three locations during the 2001–2002 season in Virginia

Constituent	Sprouts	Seeds	% Change from seed	Standard deviation
Oil (%)	27.2	39.7	–31.5**	2.48
Total sterol (g/100 g oil)	10.7	8.8	21.6**	5.53
Individual sterol (μg/g of oil)				
Brassicasterol	1,161.93	1,070.1	8.6	403
Campesterol	3,799.4	2,989.2	27.1**	604
Stigmasterol	33.5	39.5	−15.2	29
β-Sitosterol	5,359.4	4,468.9	19.9**	820
Δ^5-Avenosterol	201.1	197.7	1.7	78
Δ^7-Stigmastenol	96.8	67.2	44.1**	37
β-Sitosterol:stigmasterol (ratio)	(160:1)	(113:1)	41.5	29

**Differences between seeds and sprouts significant at the 1% level

dried sprouts (Table 2). Sprouts from seeds produced in the Petersburg location had the highest concentration of *β*-sitosterol (5,840.6 μg/g oil), campesterol (4,040.2 μg/g oil) and brassicasterol (1,250.1 μg/g oil), whereas seed produced in the Suffolk location had the lowest brassicasterol (1,023.4 μg/g oil) and *β*-sitosterol (5,109.6 μg/g oil), respectively. On the other hand, sprouts produced from seeds grown at the Suffolk and Orange locations had the highest (30.2%) and lowest (25.3%) oil content, respectively. The results demonstrated that lipid content was significantly reduced (31.5%), whereas the content of phytosterols was significantly increased (22.2%) upon sprouting over contents in seeds (Table 3). Our data also showed that oil constitutes about 40/100 g of ungerminated canola seeds, of this amount about 32% (12.5 g) is used during germination. The present findings are in agreement with Chung et al. [2], who reported losses in lipid content and increases in phytosterol during germination of both canola and barley seeds. In fact the reduction in lipids by the sprouting process of canola seeds can be attributed to their use to support growth and generation of new cells and as respiratory substrate [21]. Furthermore, our data demonstrated that *β*-sitosterol/stigmasterol ratios in sprouts and in ungerminated seeds were 160:1 and 113:1, respectively (Table 3). Apparently, owing to its planar structure as compared to stigmasterol, *β*-sitosterol plays an important role in minimizing membrane permeability [6, 22]. Therefore, a higher ratio of *β*-sitosterol/stigmasterol should maintain membrane fluidity especially at low temperatures.

The present results indicated that *β*-sitosterol, campesterol and brassicasterol were the predominant sterols and accounted for over 96% of the total sterols, whereas stigmasterol, Δ^5-avenasterol and Δ^7-stigmastenol were the minor sterols in canola sprouts and amounted to less than 4% of the total sterols. In addition, our data demonstrated significant changes in the content and composition of total sterols in canola sprouts as compared to un-germinated seeds (Table 3). Furthermore, our data indicate significant

increases in total sterols (22.2%,), campesterol (27.1%), β-sitosterol (19.9%) and Δ^7-stigmastenol (44.1%), whereas Brassica sterols (8.6%), stigmasterol (−15.2%) and Δ^5-avenosterol (1.7%) did not exhibit significant differences between seeds and sprouts (Table 3). In general, our data exhibited similar pattern with those reported on the metabolic changes of oil, total sterol and individual sterols in germinating seeds [2, 7, 23–25]. They also indicated that the total sterols increased, with β-sitosterol accounting for the major sterol whereas, stigmasterol and campesterol showed the greatest changes during germination.

It is noteworthy to mention that phytosterols are health-promoting minor bioactive phytochemicals which have hypocholesterolemic, anti-inflammatory and anti-carcinogenic effects on human health [9–13]. Consequently, the present results suggest that canola sprouts can be considered a good source for dietary sterols. Also, consumption of equal amount of oil from canola sprouts as a dietary source of plant sterols will provide about 22 and 20% more of total sterols and β-sitosterol, respectively, than those from ungerminated seeds. Therefore, consumption of dietary sterols from canola sprouts might be more beneficial to human health than those from commercial edible vegetable oils. The total percentage of sterols in fresh canola sprouts containing 5.4% oil is equivalent to about 0.06/100 g of the fresh sprouts. In contrast an edible vegetable oil with an average of 0.60% sterols is subjected to a loss about 60% of total sterols during processing, refining, deodorization, deep-frying and cooking [26–29]. This loss will decrease the amount of total sterols in processed oil to about 0.16 g in 65 g oil (Reference Daily Intakes (RDIs) of fat based on 2,000 calories a day for adults and children over 4 or more years of age [30]. This amount is equivalent to consumption of about 250 g fresh canola sprouts a day (0.15 g sterols per day). In addition to the health- promoting uses of dietary sterols, consumption of 250 gram of fresh canola sprouts will contribute to RDIs for an adult by about 21% each of fat (13.5 g), protein (12.5 g) and fiber (5.3 g) as indicated from our previous studies [3]. The oil also have the lowest amount of saturated fat (about 7% of total fatty acids) and the highest value of 18:3 (omega-3) fatty acid (about 9% of total fatty acids) as shown from our published data [31], with the highest ratio of 18:3/18:2 (1:2) among commercial edible vegetable oils [19, 20].

The oil content in canola sprouts was positively correlated with total sterols expressed as g/100 g UNSAP and Δ^5-avenasterol, whereas oil content was negatively correlated with brassicasterol (Table 4). The content of UNSAP (g/100 g of oil) was positively correlated with sterol content expressed as mg/g of oil, sterol content expressed as mg/g dry sprouts and contents of campesterol and β-sitosterol. However, the content of UNSAP (g/100 g of oil), was negatively correlated with sterol content expressed as g/100 g of UNSAP (Table 3). In general, the contents of campesterol and β-sitosterol increased with an increase in total sterol content.

Table 4 Correlations among composition traits of sterols in sprouts of seven canola cultivars grown at three locations during 2001–2002 season in Virginia

	UNSAP1[a]	UNSAP2[a]	S1[a]	S2[a]	S3
Oil	−0.43**	0.19	0.31*	−0.21	−0.16
UNSAP1		0.79**	−0.62**	0.56**	0.95**
UNSAP2			−0.46**	0.48**	0.93**
Sterols 1				0.30	−0.57**
Sterols 2					0.55**

	BS[b]	CS[b]	SS[b]	βS[b]	Δ^5-A[b]	Δ^7-S
Oil	−0.47**	0.05	−0.05	−0.22	0.33*	0.02
UNSAP1	0.27	0.48**	0.01	0.50**	0.08	0.09
UNSAP2	−0.03	0.58**	−0.05	0.41**	0.28	0.10
Sterols 1	0.25	0.11	−0.01	0.30	0.10	−0.07
Sterols 2	0.57**	0.71**	−0.04	0.94**	0.18	0.01
Sterols 3	0.14	0.71**	−0.04	0.95**	0.18	0.01
BS[b]		−0.01	0.23	0.52**	0.12	0.03
CS[b]			−0.23	0.54**	0.22	−0.03
SS[b]				−0.04	0.07	0.02
βS[b]					0.02	−0.05
Δ^5-A[b]						0.47**
Δ^7-S[b]						

*, ** Correlation coefficient significantly different from zero at the 5 and 1% level of significance, respectively

[a] *S1* total sterols g/100 g UNSAP, *S2* total sterols mg/g of oil, *S3* mg/g dry sprouts

[b] *BS* brassicasterol, *CS* campesterol, *SS* stigmasterol, *βS* β-sitosterol Δ^5-A = Δ^5-avenasterol; Δ^7-S = Δ^7-stigmastenol. All sterols expressed as µg/g of oil

We conclude that the growing locations had a greater influence on oil and sterols content in canola sprouts as compared to the effects of canola lines. These findings, however, may change when a larger number of lines are evaluated. We also concluded that canola sprouts may have potential as a natural source of dietary sterols and thus, desirable for human consumption.

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