Variation in beta -Carotene and other Antioxidants among Different Species of Oilseed Brassica

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Variation in β-Carotene and other Antioxidants among Different Species of Oilseed Brassica

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Cultivated varieties of oilseed Brassica belonging to seven different taxa were analyzed for various antioxidants to identify best lines in terms of antioxidant properties. The highest mean total antioxidant capacity (TAC) was observed in *B. carinata* group (23.12 mg/g AAE) and the lowest in *Eruca sativa* group. The highest and the lowest radical scavenging activities (RSA) were both observed in yellow sarson group. The mean value of β-carotene content was highest in the *B. carinata* (1.55 ppm) group and lowest in *Eruca sativa* (0.80 ppm) group. The lowest average ascorbic acid content was shown by *B. juncea* (51.67 mg/g) group. The mean value of total phenol content was highest in *B. carinata* (1.73 %) followed by *B. juncea* (1.68 %) and *B. napus* (1.30 %). It was found to be lowest in brown sarson group (1.17 %). *Eruca sativa* varieties showed very high levels of total flavonoids compared to other species with an average of 1160 µg/g QE. It was observed that the oil content had significant negative correlation with total flavonoids (-0.76). Hence, high flavonoid lines can be expected to have low oil content or vice versa. β-carotene has a very significant positive correlation with TAC (0.28). It may be due to the fact that β-carotene contributes the most towards antioxidant property compared to phenolic compounds or ascorbic acid. RSA was found to be negatively correlated to TAC (-0.25) which may be because antioxidant property is governed mostly by antioxidant compounds other than radical scavengers. RSA was also negatively correlated to both ascorbic acid (-0.32) as well as β-carotene (-0.23).

Key words: Brassica, β-carotene, antioxidants, ascorbic acid, phenols, flavonoids

Rapeseed-mustard crops of the genus Brassica include many cultivated species such as *Brassica juncea* (Indian mustard), *B. rapa* var. Yellow sarson, *B. rapa* var. Toria, *B. rapa* var. Brown sarson, *B. napus* (Gobhi sarson), *B. carinata* (Karan rai) and *Eruca sativa* (Taramira). In India, these crops are grown mainly for the purpose of edible oil in various agroclimatic conditions ranging from the plains of Rajasthan, Punjab and Haryana to the sub-Himalayan and north-eastern regions. Rapeseed-mustard meal has been reported to be rich source of antioxidants (1,2,3). Its antioxidant potential has been mainly attributed to the redox properties of phenolics which act as reducing agents, hydrogen donors and singlet oxygen quenchers. Compounds such as ascorbic acid and flavonoids also contribute to the antioxidant potential of seed meal. Due to this property, Brassica seed meal has been successfully used in preserving ground meat (4,5). At the same time, tocopherols, phytosterols and â-carotene contribute to the antioxidant properties of the oil fraction (6). The reason for high stability of rapeseed-mustard oils even in atmosphere conducive for oxidation is the abundance of natural antioxidants which are mostly phenolics. These components essentially provide protection for polyunsaturated fats which otherwise are unstable in nature (7).

Antioxidants are compounds that can retard oxidation of unstable materials such as unsaturated fatty acids by preventing accumulation of free radicals or reactive oxygen species (ROS). Free radicals are generated by physiological processes occurring in the body and by biotic and abiotic stresses leading to diseases (8). ROS, if not eliminated will attack biological molecules such as lipids, proteins and nucleic acids (9). In human beings such oxidative stresses have been associated to cardiovascular diseases, hypertension, cancer and other ailments (10). The antioxidant potential of certain food stuffs is directly correlated to the presence of biomolecules having predominant antioxidant activity such as polyphenols and vitamins (11). Epidemiological

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studies have shown that consumption of plant products rich in antioxidants is associated with a reduced risk of many diseases such as atherosclerosis and cancer (12,13). A mixture of polar and non-polar antioxidants is capable of quenching free radicals in both aqueous and lipid phases (14). Hence food products rich in antioxidants should be inevitable in our diet. Previous studies show that there is considerable variation in amounts of bioactive compounds such as phenolic compounds, ascorbate and â-carotene among different cultivars of rapeseed (15). Systematic research on antioxidant compounds of plant origin may result in popularization of such crops as well as to make use of them as nutraceuticals for health and well-being.

Materials and Methods

Seeds of 45 different cultivars of rapeseed-mustard including 15 varieties of *Brassica juncea* and 5 varieties each of *Brassica rapa* var. Yellow sarson, *Brassica rapa* var. Toria, *Brassica rapa* var. Brown sarson, *Brassica napus*, *Brassica carinata* and *Eruca sativa* were selected for the study. The seed samples were obtained from the germplasm collection of ICAR-DRMR during the month of April 2015. Oil content was determined gravimetrically after extracting with hexane. Total flavonoid content was estimated in methanolic extracts prepared from the seed meal using a spectro UV-Vis Double beam UVD-3500 spectrophotometer (16). Flavonoid content was expressed as quercetine equivalents. Total antioxidant activity (17), radical scavenging activity (18) and β-carotene content (AACC 14-50, 1995) were estimated by spectrophotometric methods. Ascorbic acid was estimated using the titrimetric method (19). Total phenols content was measured by non destructive method using a Bruker matrix-1 FT-NIR (20). The experiment was conducted in CRD design with three replications for each parameter. Data was analysed using a mixed model of analysis of variance (ANOVA) with species as fixed effect and genotypes nested within species as a random effect using the statistical programming language R. The species effect was tested against the random effect of genotypes nested within species. The mean differences between species were compared by Tukey's test using the R package Tuckey C. $P < 0.05$ was considered as statistically significant. Box-plots were plotted using the R package ggplot2.

Results and Discussion

β-carotene: Canola oil has been found to have β-carotene content higher than hemp and flax seed oils (21). Intake of β-carotene which is a precursor of vit-A through diet has been related to human health due to its antioxidant properties (22). Several attempts have been made to enhance β-carotene in edible oils such as suppression of de-etiolated-1 (DET-1) gene by RNAi in *B. napus* has resulted in increase of seed carotenoid accumulation (23). In the present study, the β-carotene content ranged from 0.03 ppm in yellow sarson variety YSH-401 to 2.82 ppm in NRCDR-2 which is a *B. juncea* variety (Table 1). The mean β-carotene was highest in the *B. carinata* (1.55 ppm) group and lowest in *Eruca sativa* (0.80 ppm) group. This shows that as far as β-carotene content is concerned, *B. carinata* varieties can be considered as the best source among oilseed Brassica species.

Ascorbic acid: There are many reports on abundance of ascorbic acid in Brassica foods, mainly, vegetable (24,25). We observed higher average ascorbic acid in *B. napus* (100 mg/g) group with maximum level in the variety GSL-1 (Table 1). The lowest average was shown by *B. juncea* (51.67 mg/g) group with the lowest levels in varieties RH-781 and RH-0749 (20 mg/g). Differences in L-galactono-c-lactonedehydrogenase a key enzyme in ascorbic acid biosynthesis which catalyses the oxidation of L-galactono-1,4-lactone to ascorbic acid was suspected to be the reason for differences in ascorbic acid content in different cultivars of Brussels sprouts (26). In case of soybean sprouts it has been reported that there is increase in ascorbic acid content during germination due to increased activity of GLDH (27). At the same time many have reported that germination caused decrease of total phenolic compounds in edible sprouting seeds (28,29). This shows that ascorbic acid and phenolic content are negatively correlated which is exactly what we have observed in case of mustard seed meal. This is further explained later in this report.

Total phenols: Rapeseed meal is known to have high phenolic acid ester content which causes dark color

Table 1: Oil content, total antioxidant capacity, radical scavenging activity, beta carotene, total phenols and total flavonoid content among different species and genotypes of oilseed Brassica.

Taxa	Variety	Oil content (%)	Total antioxidant capacity (mg/g AAE)	Radical scavenging activity (%)	Ascorbic acid (mg/g)	Beta carotene (ppm)	Total phenol (%)	Total flavonoids (µg/g)
Brassica juncea	RB 50	41	12.95	45.38	40	0.98	1.69	733.33
	RH 781	40.77	18.29	47.48	20	0.98	1.52	639.22
	RH 819	41.5	20.95	44.12	40	1.39	1.87	568.63
	RH 0406	39.22	17.79	47.48	60	2.00	1.41	662.75
	RH 0749	39.46	21.41	43.28	20	1.28	1.52	815.69
	NRCDR 2	40.45	17.41	46.64	60	2.82	1.54	611.76
	NRCHB 101	40.14	16.54	44.96	60	0.06	1.81	674.51
	DRMR IJ-31	39.17	16.66	46.64	60	0.33	1.50	576.47
	Swarna	42.52	20.25	43.28	60	0.09	1.78	631.37
	NPJ-112	40.5	21.95	32.77	40	0.99	1.91	600.00
	DRMR-1288	41.22	15.91	40.76	60	0.43	1.72	745.10
	Basanti	41.58	18.00	40.34	80	0.95	1.76	686.27
	CS-52	41.05	11.87	39.08	15	0.95	2.09	737.25
	RCC-4	40.41	13.79	40.34	120	0.99	1.54	741.18
	RGN-73	40.52	18.66	40.34	40	0.98	1.54	776.47
Brassica rapa var. yellow sarson	YSH 401	44.8	14.04	49.16	60	0.03	1.33	443.14
	NRC YS 5-2	42.19	27.41	41.18	40	0.66	1.33	517.65
	Ragini	43.08	27.29	23.53	80	1.43	1.41	443.14
	Jhumka	43.74	23.45	35.29	100	1.31	1.32	486.27
	Benoy	42.85	14.12	32.77	60	1.32	1.29	474.51
Brassica rapa var. Toria	Bhavani	43.23	13.62	31.93	60	0.07	1.2	541.18
	PT 303	42.53	6.66	35.71	40	0.11	1.12	705.88
	PT-30	40.47	31.54	37.39	60	1.43	1.29	658.82
	TS-36	40.54	18.29	34.87	180	1.26	1.22	576.47
	Anuradha	40.86	18.83	38.66	80	1.21	1.17	705.88
Brassica rapa var. Brown Sarson	KBS 3	41.25	29.50	37.39	60	0.38	1.05	607.84
	KOS 1	41.19	9.50	37.39	60	0.25	1.13	807.84
	DRMR-82	40.26	21.83	39.50	60	2.00	1.75	705.88
	DRMR-973	41.18	12.83	34.45	100	0.79	1.15	623.53
	DRMR-388	39.08	34.33	36.97	40	0.62	0.75	537.25
Brassica napus	GSL 1	42.38	34.70	31.93	200	0.19	1.14	725.49
	Sheetal	41.66	28.70	32.77	60	0.66	1.36	725.49
	Neelam	39.07	13.12	36.97	60	0.70	1.41	564.71
	GSC-5	39.66	15.87	28.57	100	1.31	1.28	745.10
	GSL-2	40.41	22.83	31.09	80	1.92	1.33	670.59
Brassica carinata	Kiran	39.42	18.16	27.31	55	0.95	1.52	678.43
	PC 5	41.19	32.91	25.21	60	2.18	1.50	839.22
	Pusa Aditya	40.78	26.45	39.08	60	1.03	2.09	749.02
	PC5-17	37.88	32.91	25.21	60	2.18	1.63	839.22
	Pusa Swarnim	41.12	5.16	26.05	80	1.43	1.89	713.73
Eruca sativa	RTM 314	38.06	9.37	40.34	80	0.18	1.48	945.10
	T 27	38.69	17.33	44.54	40	0.31	1.21	1066.67
	DRMR-171	36.79	26.66	39.50	60	1.63	1.08	1121.57
	DRMR-173	37.04	19.75	37.39	40	1.58	1.44	1243.14
	TMLC-2	35.37	13.45	39.92	40	0.29	1.19	1423.53

and bitter taste. Phenolic content of rapeseed is higher than any other oilseed plant (30) almost 30 times that of soybean (15) and it has been reported to be within the range of 1080.2-1807 mg/100 g (31). Sinapic acid is the most common phenolic acid in rapeseed having free radical scavenging property (32). Tannins are other important phenolic compounds that are present in abundance in rapeseed-mustard meal fraction. Most of them are condensed tannins formed by polymerization of flavan-3-ols or flavan-3,4-diols (33). Tannins isolated from canola are natural antioxidants and have DPPH radical scavenging activity of 35.2 to 50.5 % (34). The rapeseed meal polyphenolic fractions are also composed of flavonols and hydroxycinnamic derivatives, which are regarded as potent free radical scavengers (35). In this study, the highest phenol content was observed in the *B. juncea* variety CS-52 and *Eruca sativa* variety Pusa Aditya (2.09 %) and the lowest in DRMR-388 (0.75 %) a brown sarson variety (Table 1). The mean value was highest in *B. carinata* (1.73 %) followed by *B. juncea* (1.68 %) and *B. napus* (1.30 %) (Table 1). The mean value was lowest in the brown sarson group (1.17 %). The results are in agreement with the earlier reports that regard genotype as the most important factor determining the levels of flavonoids in foods (36).

Total flavonoids: Flavonoids are low molecular weight phenolics that can be divided into subclasses according to the structure of the flavone skeleton, variation in position of hydroxyl group and substitutions (37). In rapeseed, the most common flavonoids are kaempferol, quercetin, isorhamnetin and myricetin (38) which are reported to have health benefits (39). For example, quercetine has been reported to have anticancer activities and inhibited cancer cell growth (40). They have also been found to prevent low-density lipoproteins and DNA oxidation by scavenging peroxyl and hydroxyl radicals (41). They are also known to have antiinflammatory and antiviral (42) properties. There was a considerable difference in total flavonoid content among different cultivars of rapeseed and values ranged from 18.14 to 32.57 mg/g (15). In our study, the highest total flavonoid content was recorded by TMLC-2 an *Eruca sativa* variety (Table 1). In fact all *Eruca sativa* varieties showed very high levels of total flavonoids compared to other species. They had an average value of 1160 µg/g QE. The lowest flavonoid content was observed in yellow sarson varieties YSH-401 and ragini (443.14 µg/g QE). The mean value for yellow sarson varieties was 472.94 µg/g QE.

Total antioxidant capacity (TAC): Ethanolic extract of canola meal have been shown to have very high antioxidant activity equivalent to that of tert-butyl hydroquinone (TBHQ) and stronger than butyl hydroxyanisole (BHA), butyl hydroxytoluene (BHT) and BHA/BHT/monoacylglyceride citrate (MGC) which are common antioxidant standards (33). Addition of 0.5-5 % canola flour has been shown to prevent 73-97 % fat oxidation in meat (43). We observed that out of 45 genotypes analyzed, the *B. napus* variety GSL-1 recorded the maximum TAC (34.70 mg/g AAE) while *B. carinata* variety Pusa swarnim showed lowest TAC (5.16 mg/g AAE) (Table 1). The highest mean TAC was observed in *B. carinata* group (23.12 mg/g AAE) and the lowest in *Eruca sativa* group.

Radical scavenging activity (RSA): Reduction in DPPH absorption is indicative of the capacity of a sample to scavenge free radicals and this method has been commonly employed for estimating antioxidant potential in terms of RSA. In a previous report, the radical scavenging activity using DPPH method varied from 64.2 to 47.03 % in Brassica seeds (15). We have observed that the highest as well as lowest RSA was recorded in yellow sarson group (Table 1). The variety YSH-401 (49.16 %) had the highest RSA and Ragini (23.53 %) showed the lowest. The mean RSA was highest in *B. juncea* group (42.86 %) and lowest in *B. carinata* group (28.57 %).

Oil content: Highest oil content of 44.8 % was recorded by YSH-401 (Table 1) which is a yellow sarson variety while the lowest was recorded by TMLC-2 (35.37 %) an *Eruca sativa* variety among the 45 genotypes. Yellow sarson varieties showed the highest mean oil content (43.33 %) followed by toria (41.53 %), *B. napus* (40.64 %) and *B. juncea* (40.63 %) (Table 1). *Eruca sativa* group had the lowest average oil content (37.19 %).

Correlation analysis: Good correlation between the content of phenolic compounds and antioxidant potential of seed meal has been reported. Also, flavonoids in oilseeds correlate with antioxidant activity due to the presence of phenolic hydrogens which act as radical scavengers (45). In this study, it was observed that the oil content had significant negative correlation with total flavonoids (-0.76) (Table 3). Hence, high flavonoid lines can be expected to have low oil content or vice versa as evident in the case of *Eruca sativa* group. Oil content was found to be positively correlated to ascorbic acid (0.20) and negatively correlated to â-carotene (-0.19). β-carotene had a very significant correlation with TAC as well (0.28).

Hence, it may be assumed that β-carotene contributes

Table 2: ANOVA showing significant variation in oil content, total antioxidant capacity, radical scavenging activity, beta carotene, total phenols and total flavonoid content among different species and genotypes

Source	df	Oil content	Total antioxidant capacity	Radical scavenging activity	Ascorbic acid	Beta carotene	Total phenol	Total flavonoids
Taxa	6	50.561***	132.02	513.83**	5755.6	1.0606	1.1031**	682213**
Variety	38	3.772**	168.00**	65.86**	3265.5**	1.3907**	0.1171**	25725**
Residuals	90	0.0001	0.000099	0.0001	1.0	0.0001	0.0001	0.0001

Table 3: Correlation between oil content, total antioxidant capacity, radical scavenging activity, beta carotene, total phenols and total flavonoid content

	Oil content	Total antioxidant capacity	Radical scavenging activity	Ascorbic acid	Beta carotene	Total phenols	Total flavonoids
Oil content	1						
Total antioxidant capacity	-0.02	1					
Radical scavenging activity	-0.08	-0.25**	1				
Ascorbic acid	0.20*	0.12	-0.32**	1			
Beta carotene	-0.19*	0.28**	-0.23**	-0.01	1		
Total phenols	0.07	-0.18*	0.12	-0.25**	0.19*	1	
Total flavonoids	-0.76**	-0.08	0.05	-0.17	0.02	-0.05	1

the most towards the antioxidant property compared to phenolic compounds or ascorbic acid. Surprisingly, the RSA was found to be negatively correlated to TAC (-0.25). It can be assumed that the antioxidant property is governed mostly by antioxidant compounds other than radical scavengers. It was also surprising to observe that RSA was negatively correlated to both ascorbic acid (-0.32) and β-carotene (-0.23). Also, ascorbic acid was negatively correlated to total phenols (-0.25).

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