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Research article

Changes in nutraceutical attributes in soybean raised at varying growing temperature

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Abstract: Investigations concerning the influence of growing temperature on the accumulation of nutraceutical traits in soybean seeds are limited. In the present study, 3 soybean genotypes were raised at 24ºC, 28ºC, and 32ºC; and the freshly harvested seeds were assessed for tocopherol isomers content, vitamin E activity, fatty acid composition, isoflavones content, and thiobarbituric acid (TBA) number. All the genotypes exhibited a sharp increase in α tocopherol, with a concomitant decline in γ tocopherol, at 32ºC. Further, lower levels of α-linolenic acid and TBA number were observed at 32ºC. Changes in the levels of daidzein, genistein and glycitein due to the increase in growing temperature were genotype-dependent. The results showed an increase in the nutraceutical value of soybean raised under elevated temperature.
Keywords: Soybean - Growing temperature - Tocopherols - Fatty acid composition - Isoflavones - TBA value.

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INTRODUCTION

Soybean has earned the sobriquet of the 'functional food of the 21st century' due to the presence of nutraceutical molecules, namely, tocopherols and isoflavones, apart from the basic nutrients, in its grains (Kumar *et al.* 2010). Tocopherols have been reported to reduce the onset of oxidative-stress related diseases like Parkinson, Alzheimer, and diabetes (Kamal-Eldin & Appelqvist 1996, Nordentoft *et al.* 2008); while the isoflavones, besides possessing weak antioxidative activity, keep breast/uterus cancer and osteoporosis *etc.* at bay (Messina *et al.* 1994, Potter *et al.* 1998). Further, unsaturated fatty acids, namely, oleic acid, linoleic acid and α-linolenic acid in soybean seeds are prone to oxidation in the ratio of 1:10:25 (Liu 2014). Soybean genotypes with the high level of oleic acid and low levels of α-linolenic acid can yield better oxidative stability of the oil extracted from the seeds, and even the flour milled from such genotypes also has a relatively better shelf life (Gorbet & Knauft 1997, Bolton & Sanders 2002). Tocopherol, in soybean seeds, exists in its 4 isomeric forms, namely, α, β, γ and δ, with free radical scavenging activity in the ratio of 1.0, 0.5, 0.1 and 0.03, respectively. Isoflavones exist in 3 major aglycon forms, namely, daidzein, genistein, glycitein.

Rise in global temperature is a serious concern with regard to the performance of the crops in terms of the grain yield and levels of the traits of economic importance. Yield and yield components of soybean crop have been reported to be affected at increased growing temperature (Bhardwaj *et al.* 1999, Puteh *et al.* 2013, Zhang *et al.* 2016). However, limited studies have been carried out in soybean concerning the effects of the increased temperature during crop growth on the accumulation of tocopherols and isoflavones, which influence the quality and nutraceutical value of the processed products. Chennupati *et al.* (2011) studied the effect of high-temperature stress at different developmental stages on soybean tocopherols concentration. This study was carried out in only one genotype. Lanna *et al.* (2005) investigated the effect of growing temperature on the accumulation of polyunsaturated fatty acids in soybean seeds. Moreover, TBA number, which is the function of polyunsaturated fatty acids and anti-oxidative potential of soybean grains and contributes to the nutritive value of the products processed from them, has not yet been assessed in soybean crop raised under varying temperature. Therefore, in the present study, 3 soybean genotypes were raised under 3 different temperature

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from sowing to harvesting, and the harvested seeds from each of the treatment were subjected to estimation of fatty acid composition, TBA number, tocopherol isomers, and isoflavones contents.

MATERIALS AND METHODS

Materials

Two Indian soybean cultivars, namely, JS20-29 and JS71-05 and one germplasm accession EC456548 were raised in pots in triplicate in 3 greenhouses, each of 6 × 3 meter size, at ICAR-Indian Institute of Soybean Research, maintained at day/night temperature of 30/18ºC, 34/22ºC, 38/26ºC, with average temperature of 24ºC, 28ºC and 32ºC, respectively. On maturity, soybean seeds of 3 genotypes from the respective growing temperature were harvested and subjected to following analysis.

Extraction and Determination of Tocopherols Using HPLC

Oil from the finely ground soy flour (30 mesh size) was extracted by soaking in HPLC-grade petroleum ether for 8 h at room temperature. The mixture was transferred into vials and the solvent was evaporated under vacuum at 30ºC. The oil in each vial was determined gravimetrically and re-dissolved the samples in fixed volume of HPLC grade n-hexane. The weight of the oil in each vial was determined gravimetrically and the samples were re-dissolved in fixed volume HPLC grade n-hexane. Tocopherols composition was determined using a Shimadzu HPLC system equipped with a UV detector and a silica-NH_2 column (Inert Sustain 5µm; 4.6 × 250 mm C/N 5020-16628). Syringe-filtered sample (20 µl) was injected into the column and eluted isocratically with HPLC-grade n-hexane and ethyl acetate (70:30 v/v) at a flow rate of 0.5 ml min^{-1}. Tocopherols were detected with UV detector (SPD 10 AT *vp*) at a wavelength of 295 nm. The resolution of the tocopherol isomers and the relative amounts of tocopherols were calculated by comparing their peak areas with a standard curve generated using different amounts of external standards of α, β, γ and δ tocopherol (Sigma-Aldrich, India). Tocopherols were expressed as µg g^{-1} oil basis and total tocopherols content was computed by summing up the values of all the 4 isomers.

Computation of Vitamin E Activity

The vitamin E activity of soybean oil from different genotypes was taken as the sum of multiplication of α, β, γ and δ tocopherol content by 1.0, 0.5, 0.1, and .03, respectively, as previously reported (Sheppard *et al.* 1993). Considering the guidelines of United States Pharmacopeia, according to which 1 mg of α tocopherol is equivalent to 1.49 International Unit of vitamin E, the value obtained as µg g^{-1} of oil was multiplied by 0.149 for conversion into International Units per 100 g of oil.

Fatty Acid Composition

Oil from finely ground soy flour (30 mesh) of each soybean genotype was extracted with 180 ml hexane in an automated Soxhlet unit (Pelican Equipments, Chennai, India) for 3 h. For fatty acid analysis, soybean oil was extracted by incubating the vial containing the mixture of soy flour and petroleum ether (boiling point 30–50ºC) at 40ºC. Fatty acid methyl esters, prepared using 1N sodium methoxide, were estimated through Gas Chromatography using Shimadzu GC17A, fitted with capillary column (SGEBPX70).

Determination of TBA number

TBA number (Lipid peroxidation) was measured as the amount of thiobarbituric acid reactive substances (TBARS) determined by the thiobarbituric acid reaction (Heath & Packer 1968). The TBA number was expressed in µmol/g.

Estimation of isoflavone isomers

Isoflavones in soybean seeds were estimated through the method given by Vyn *et al.* (2002). The acid hydrolysis of soy flour samples was carried out to determine the contents of isoflavones isomers, namely, diadzein, glycitein and genistein as described elsewhere (Kumar *et al.* 2011). Contents of isoflavones isomers, namely, daidzein, glycitein and genistein were determined through acid hydrolysis of soy flour samples as described elsewhere (Kumar *et al.* 2011). The method converts 12 endogenous isoflavone isomers to their respective aglycone forms *i.e.* daidzein, glycitein and genistein.

Statistical Analyses

All the statistical analyses were carried out using SAS *version* 9.3.

RESULTS AND DISCUSSION

Tocopherols and Vitamin E activity

Table 1 depicts the contents of 4 isomers of tocopherols, and Vitamin E activity in the oil fraction of seeds of

3 genotypes grown at 24ºC, 28ºC, and 32ºC. With regard to α isomer, the significantly higher levels were accumulated at 32ºC than 24ºC and 28ºC in all the 3 genotypes. An increase in α isomer occurred with a concomitant decline in γ isomer. Though, the magnitude of increase in the concentration of α isomer and decline in γ isomer at 32ºC than 24ºC was genotype-dependent, as indicated by 72.9, 70.2, and 55.6% higher accumulation of α-isomer and 31.0, 32.3, 49.2 % decline in γ isomer at 32ºC than 24ºC in JS20-29, JS71-05 and EC456548, respectively. In developing seeds, α isomer is synthesized from γ isomer by gamma methyl transferase activity (Lushchak & Semchuk 2012). The increased synthesis of α isomer and lower accumulation of gamma isomer at higher growing temperature may be attributed to the high activity of gamma methyl transferase at higher growing temperature. In general, β-isomer did not change significantly at higher temperatures except in EC456548, which registered an increase (29.5%) at 32ºC than 24ºC. Further, accumulation of δ tocopherol was significantly ($P < 0.05$) higher at 28ºC and 32ºC than at 24ºC in all the 3 genotypes, though the magnitude of increase due to the increase in temperature in this isomer also was genotype-dependent, as evident from 27.6, 68.2, 39.1% higher accumulation for this isomer at 32ºC than at 24ºC in JS20-29, JS71-05 and EC456548, respectively. These changes in the tocopherol isomers at varying growing temperature, resulted in significantly ($P < 0.05$) higher vitamin E activity at 32ºC than at 24ºC and 28ºC in all the 3 genotypes. JS20-29, JS71-05 and EC456548 registered 100.6, 137.0, and 73.8% higher vitamin E activity at 32ºC than at 24ºC. Though, no significant differences were noted for vitamin E activity in all the 3 soybean genotypes grown at 24ºC and 28ºC. Chennupati *et al.* (2011) also studied the effect of temperature stress at different developmental stages of soybean on tocopherol isomers. A significant increase in α tocopherol at 29ºC than at 19ºC reported by these authors supports our results, though the 10–15 fold increase for this isomer in their study was far higher than 2.0–2.5 folds observed in our investigation. This difference in the magnitude of increase for α isomer maybe because of the much lower temperature (19ºC) in the study of Chennupati *et al.* (2011) than in our investigation in the present investigation (24ºC). Carrera *et al.* (2011) also observed higher α-tocopherol at the higher temperature (22.5–25.0ºC) compared to lower temperature (17.5–22.4ºC). Increase in accumulation in tocopherols isomers at high growing temperature noted in our results and the previous studies (Carrera *et al.* 2011, Chnnupati *et al.* 2011) may be due to the response of the crop plant to adapt to temperature stress as suggested in the earlier study (Britz & Kremer 2002).

Table 1. Tocopherol, vitamin E activity and isoflavones content of soybean seeds of different genotypes harvested at varying temperatures.

Genotype	Temp. (ºC)	Tocopherols (μg g^{-1} oil)				Vitamin E (IU/100 g oil)	Isoflavones (μg g^{-1} soy flour)		
		α	β	γ	δ		Daidzein	Genistein	Glycitein
JS20-29	24	100.84± 9.4^{e}	87.64± 7.9^{a}	1316.2±12.1^{a}	101.9±8.4^{e}	32.40±2.9^{d}	178.0±15.2^{f}	404.5± 38.0^{g}	155.6±14.2^{e}
	28	122.82± 10.8^{d}	75.21± 3.1^{a}	1261.5±10.2^{a}	145.37±13.2^{c}	32.29±3.1^{d}	234.5±21.0^{e}	455.0± 42.1^{f}	144.3±13.1^{e}
	32	373.06± 32.8^{b}	76.63± 5.2^{a}	907.74±85.1^{bc}	140.91±12.0^{c}	65.0±5.8^{b}	309.5±30.0^{c}	455.0± 43.0^{fg}	149.6±12.1^{e}
JS71-05	24	140.04± 12.6^{d}	58.95± 4.3^{bc}	977.69±89.2^{b}	75.67±6.8^{f}	32.53±2.8^{d}	491.0±42.3^{a}	873.5± 85.1^{a}	448.0±40.2^{a}
	28	98.48± 7.8^{e}	59.12± 4.4^{bc}	864.43±82.0^{c}	124.91±11.4^{d}	25.74±2.1^{d}	419.6±38.1^{b}	667.3± 65.0^{d}	344.0±32.2^{c}
	32	471.51± 42.3^{a}	61.66± 5.9^{b}	662.33±63.0^{d}	238.03±21.1^{a}	76.42±6.9^{a}	301.9±28.1^{cd}	385.5± 35.1^{g}	306.5±28.1^{d}
EC456548	24	167.61± 14.2^{c}	35.73± 2.8^{d}	934.62±90.1^{bc}	114.78±10.8^{de}	34.92±1.9^{d}	243.0±22.1^{e}	800.0± 58.1^{e}	391.8±37.1^{b}
	28	161.37± 14.1^{c}	35.02± 3.1^{d}	789.62±72.3^{c}	148.94±12.3^{c}	32.67±2.4^{d}	281.5±24.1^{d}	797.0± 77.1^{b}	429.7±40.1^{a}
	32	377.91± 33.3^{b}	50.67± 4.7^{c}	474.33±43.2^{e}	188.41±16.2^{b}	60.73±3.7^{c}	188.0±17.2^{f}	747.0± 7 2.1^{c}	359.1±31.1^{c}

Note: Values given are mean of 3 replicates ± standard deviation. Values within the same column with different superscripts are significantly different from each other at $p < 0.05$.

Isoflavones

As evident from Table 1, the response for changes in isoflavones isomers at different growing temperature was genotype-dependent. In general, at high growing temperature of 32ºC, significantly low values were observed for daidzein, glycitein and genistein in JS71-05 and EC456548 than at 24ºC. The decline in the levels of isoflavones in these genotypes was in consonance with the study of Chennupati *et al.* (2011) who also reported drastic reduction in total isoflavones content when growing temperature was increased from 23ºC/15ºC (day/night) to 33ºC/25ºC (day/night). Conversely, JS20-29 registered significantly ($P < 0.05$) higher value for daidzein and genistein at 32ºC than at 24ºC, while no change was noted for glycitein in this genotype at different temperatures.

TBA number (Thiobarbituric Acid Assay) and fatty acid composition

Table 2 indicates the TBA number and fatty acid composition of 3 soybean genotypes raised at 3 different temperatures. TBA number measures the oxidation of unsaturated fatty acids. In any biological system, reduction in TBA number indicates the improvement in the TBA value. Among 3 unsaturated fatty acids, 2 polyunsaturated fatty acids, namely, linoleic and α-linolenic acid oxidize 10 and 25 times faster than

monounsaturated oleic acid, respectively. Apart from antioxidant enzymes, antioxidant molecules like tocopherol isomers prevent the oxidation of fatty acids. With regard to fatty acid composition, our results showed that soybean genotypes, in general, registered significantly lower values for α-linolenic and higher values for oleic acid at 32ºC than at 24ºC. Further, soybean genotypes showed a tendency for lower TBA number at 28ºC and 32ºC than at 24ºC. In JS20-29, only slight difference for TBA number was noted for this genotype at 24ºC and 28ºC; though, significantly ($P<0.05$) less TBA number was noted at 32ºC than at the lower temperatures (24ºC and 28ºC). In JS71-05, TBA number noted at both 28ºCand 32ºC was significantly ($P <0.05$) less than at 24ºC. EC456548 did not show significant ($P<0.05$) difference in TBA number at 24ºC and 28ºC; and even at 32ºC only a slight reduction in TBA number was noted for in this genotype. This improvement in TBA value in the seeds harvested from the plants grown at the high temperature noted in our results may be attributed to the decline in α-linolenic acid with the concomitant increase in total tocopherols content and vitamin E activity at higher growing temperature.

Table 2. Fatty acid content and TBA number of soybean seeds of different genotypes harvested at varying temperatures.

Genotype	Temp (ºC)	Fatty acid (%)					TBA number (µmol g^{-1})
		Palmitic	Stearic	Oleic	Linoleic	Linolenic	
JS20-29	24	13.03±1.0^{a}	3.63±0.1^{a}	28.24±1.1^{c}	47.52±2.3^{b}	6.88±0.2^{b}	45.9±2.0^{cd}
	28	12.30±0.9^{ab}	3.38±0.2^{ab}	21.6±1.02^{e}	54.04±3.1^{a}	7.20±0.4^{b}	47.8±2.8^{bc}
	32	10.28±0.8^{b}	2.95±0.09^{b}	44.20±2.1^{a}	37.76±2.1^{c}	4.78±0.1^{c}	38.09±1.2^{e}
JS71-05	24	11.22±0.0^{b}	3.26±0.9^{ab}	23.12±1.6^{de}	54.21±3.1^{a}	8.16±0.1^{a}	53.41±3.8^{a}
	28	11.3±0.7^{b}	3.36±0.8^{ab}	24.28±1.9^{d}	54.18±3.7^{a}	6.88±0.2^{b}	46.91±2.9^{c}
	32	11.71±0.9^{ab}	3.60±0.2^{a}	23.70±1.9^{de}	54.42±4.1^{a}	6.38±0.3^{c}	44.59±2.9^{d}
EC456548	24	9.96±0.6^{b}	2.96±0.1^{b}	30.81±2.9^{b}	47.13±2.4^{b}	8.42±0.4^{a}	49.2±3.0^{b}
	28	10.33±0.7^{b}	3.02±0.1^{ab}	31.09±3.0^{b}	48.50±2.9^{b}	7.03±0.3^{b}	49.2±2.8^{b}
	32	10.20±0.1^{b}	2.93±0.1^{b}	30.35±2.2^{bc}	49.93±3.8^{b}	6.37±0.3^{c}	47.8±2.5^{bc}

Note: Values given are mean of 3 replicates ± standard deviation. Values within the same column with different superscripts are significantly different from each other at $p <0.05$.

CONCLUSION

To sum up, biochemical traits of soybean grains which determine both the quality as well as the nutritional/nutraceutical value of the final products processed from them are a function of the temperature under which the crop is raised. In the present study, the levels of tocopherols and isoflavones in soybean grains were found to be influenced by the température during crop growth. Results have shown that high growing temperature (32ºC) enhanced the TBA value of harvested soybean seeds, which may be attributed to the increase in tocopherol isomers/vitamin E activity in tandem with decline in α-linolenic acid. However, the changes in isoflavones content at different growing temperatures were genotype-dependent. The study suggests that soybean grains harvested from the crop raised under high temperature may have higher nutaceutical value.

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