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Fatty Acid Composition of Developing Soybeans

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Fatty acid composition and total free fatty acid content of seven soybean [*Glycine max* (L.) Merr.] cultivars at various stages of bean development from the 45th day after flowering up to the 75th day, at 10-day intervals, were determined. In almost all the cultivars amounts of palmitic, stearic, and oleic acids and total free fatty acids decreased while those of linoleic and linolenic acids increased during seed maturation. Minor variability in the pattern of these components in different cultivars of this legume was observed.

Soybean [*Glycine max* (L.) Merr.] is used mainly for oil extraction and meal production as it is a chief source of oil and protein (Smith and Circle, 1972). Extensive investigations on complete chemical composition of developing seeds of this legume have been carried out (Yazadi-Samadi et al., 1977; Yao et al., 1983). However, detailed information on its fatty acid composition is rather scanty (Roehm and Privett, 1970; Rubel et al., 1972; Privett et al., 1973; Manek, 1975). Further, investigations on the comparative changes in fatty acid composition of different cultivars of soybean during bean maturation have so far not been conducted. The objective of this paper is, thus, to examine the extent of variability in fatty acid composition of different cultivars of soybean during seed development. Such information on genetic variability in fatty acid composition may be useful in future efforts to effect quantitative and qualitative improvement in oil content of this legume.

MATERIALS AND METHODS

Seven commercial cultivars of soybean, namely Alankar, Ankur, Black tar, Bragg, Cobb, HM-1 and SH-3 were used in the present studies. The soybean crop was raised in the experimental farm of Haryana Agricultural University, Hissar, India, during the 1982 growing season. The flowers were tagged immediately after their emergence. The pod samples were harvested at intervals of 10 days from the 45th day after flowering, and the sampling was continued up to the 75th day when the crop matured.

Immediately after harvest, the pods were shelled and the collected seed samples were dried at 50 °C in a hot-air oven for 48 h. The dried seeds were ground to a fine powder and extracted with petroleum ether at ambient temperature. The petroleum ether extract was filtered and concentrated in vacuo below 40 °C. The extracted oil was kept in air-tight bottles and stored in a refrigerator until further analysis.

Total free fatty acid content was estimated by titrating the oil sample against standard alkali by AOAC method No. 28.032 (1984), and the results are expressed as equivalent to oleic acid.

Methyl esters of fatty acids were prepared from the oil by transesterification in methanol using sodium methoxide as catalyst (Luddy et al., 1968). Fatty acid methyl esters were separated by GLC using an Aimil Nucon gas chromatograph series 5500 fitted with flame ionization detector and stainless-steel column ($^1/_8$ in. o.d. × 8 ft) packed with 15% polydiethylene glycol succinate on Chromosorb W under standard operating parameters (Gupta and Dhindsa, 1982). The components were identified by comparison of their retention times with those of authentic samples recorded under the same operating parameters. The peak areas were calculated by multiplying peak height with width at half-height and were normalized to relative area percents (McNair and Bonelli, 1969).

RESULTS

The changes in the relative area percents of methyl esters of constituent fatty acids during seed development in the different cultivars of soybean are presented in Table I. A perusal of the data indicates that saturated fatty acids, palmitic and stearic acids, decreased as the seed matured in all the cultivars except Bragg and SH-3, which showed slight increase in palmitic acid during advanced stages of maturity. However, the rate of decrease in palmitic acid content in other cultivars at advanced stages of maturity was much lower as compared to that at earlier stages. Oleic acid content showed a slight increase at initial stages in Alankar, Ankur, and Black tar and then decreased at later stages. In other cultivars studied oleic acid content decreased progressively with seed development. Linoleic acid content increased in all cultivars with advancement of seed maturity. Except in Alankar and Ankur, linolenic acid increased in all other cultivars with seed maturation. In Alankar, it initially increased and later on decreased while in Ankur it remained almost unchanged. Total free fatty acid content in all cultivars decreased with advancing maturity of seeds.

DISCUSSION

The fatty acid composition of soybean oil has been reported by other workers as follows (percent): palmitic,

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Table I. Changes in the Relative Peak Area Percents of Methyl Esters of Constituent Fatty Acids in Soybean Cultivars during Seed Development

cultivar	days after flowering	rel peak area (% methyl esters)					total free fatty acids as oleic acid, %
		palmitate	stearate	oleate	linoleate	linolenate	
Alankar	45	14.9	2.3	25.1	44.3	13.4	1.9
	55	10.0	2.2	25.8	43.9	18.1	1.5
	65	10.0	2.1	26.2	46.4	15.3	1.1
	75	9.9	1.9	20.0	53.2	15.0	0.8
Ankur	45	14.7	3.0	25.8	42.3	14.2	2.4
	55	10.2	2.8	25.8	46.2	15.0	2.1
	65	9.5	2.1	25.0	48.8	14.6	1.7
	75	9.6	1.9	20.7	53.0	14.8	1.3
Black tar	45	15.3	4.8	26.9	40.2	12.8	1.9
	55	10.7	3.5	27.2	44.4	15.1	1.5
	65	7.7	3.1	23.1	48.2	17.9	1.3
	75	7.7	2.9	21.7	49.7	18.0	1.2
Bragg	45	19.7	2.6	29.0	36.3	12.4	1.3
	55	8.9	2.1	27.7	47.9	13.4	1.0
	65	8.1	2.0	24.2	50.2	15.5	0.8
	75	10.3	2.2	20.7	51.1	15.7	0.6
Cobb	45	15.8	5.2	35.5	34.4	9.1	1.7
	55	10.1	4.9	28.0	44.2	12.8	1.5
	65	9.4	4.3	24.0	48.8	13.5	1.2
	75	8.9	4.1	20.9	50.6	15.5	0.8
HM-1	45	17.3	5.9	25.3	39.0	12.5	1.8
	55	13.1	5.7	23.5	42.3	15.4	1.4
	65	11.0	5.4	21.8	46.2	15.6	1.1
	75	9.4	5.4	16.5	49.3	19.4	0.7
SH-3	45	16.3	3.9	30.8	39.2	9.8	1.9
	55	11.2	3.4	29.0	43.7	12.7	1.6
	65	9.1	2.8	23.8	49.7	14.6	1.4
	75	9.4	2.5	18.5	52.9	16.7	1.1

9.3–17.4; stearic, 2.2–7.0; oleic, 15.2–29.6; linoleic, 33.8–59.6; linolenic, 4.3–15.0 (Lang, 1973). All the cultivars examined in the present studies, however, contained somewhat higher levels of linolenic acid. Further, our results showed a decrease in levels of oleic acid and an increase in those of linolenic acid with advancement of seed maturity and are in contrast to those reported by others (Roehm and Privett, 1970; Rubel et al., 1972; Privett et al., 1973; Manek, 1975). The differences in results regarding changes in oleic and linolenic acid contents, in addition to varietal differences, may be due to agroclimatic factors as it is known that the enzymes involved in the fatty acid biosynthesis in soybean are influenced by both environmental and inheritance characteristics (Howell and Collins, 1957; Collins and Sedwick, 1959; Singh and Hadley, 1968; Brim et al., 1968; Hammond et al., 1972; Howell et al., 1972). The decrease in levels of palmitic and stearic acids and increase in that of linoleic acid with seed maturity are in agreement with the observation of the other workers. This pattern of change is expected as the short-chain saturated acids are believed to be the intermediates in biosynthesis of higher unsaturated acids (Kannangara et al., 1973) rather than the alternative pathway suggested earlier (Stearns, 1971) that involves desaturation of linoleic acid. However, as oleic acid content also decreases, from the changes in the gross levels of these fatty acids, it is not possible to distinguish the precise pathway for biosynthesis of unsaturated fatty acids in developing soybeans.

Total free fatty acid content in all the cultivars under study decreased as the beans become more mature as is reported by others also (Melvin et al., 1953; Feka et al., 1971; Urbanski et al., 1980; Yao et al., 1983). The result is expected since the free fatty acids are being utilized for oil synthesis during seed maturation (Yao et al., 1983).

The fatty acid composition data of the oil from developing beans indicate that oil of mature seeds is superior to that of immature ones as it contains low amounts of free fatty acids and higher amounts of essential unsaturated fatty acids, namely linoleic and linolenic acids. The usefulness of these unsaturated fatty acids has been recognized in various CVS and CNS disorders and as precursors for prostaglandins, a group of hormones that regulates vital body functions (Cowan, 1973).

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Registry No. Palmitate, 57-10-3; stearate, 57-11-4; oleate, 112-80-1; linoleate, 60-33-3; linolenate, 463-40-1.

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Effect of Processing on Available Carbohydrates in Legumes

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The effect of various treatments such as (i) soaking in plain water and sodium bicarbonate solution, (ii) cooking of soaked seeds, (iii) autoclaving of soaked seeds, (iv) germination, and (v) frying of germinated seeds, commonly employed to destroy the flatulence factors in legumes, was investigated on available carbohydrates of Rajmah (*Phaseolus vulgaris*), Bengal gram (*Cicer arietinum*), black gram (*Phaseolus mungo*), red gram (*Cajanus cajan*), and broad bean (*Vicia faba*). Total soluble sugars, reducing sugars, nonreducing sugars, and starch content in above pulses ranged from 7.09 to 10.33%, 0.18 to 0.83%, 6.91 to 9.60%, and 43 to 53%, respectively. The contents of all these components decreased under various treatments. However, on germination for 24 h, the losses in the amount of total sugars, reducing sugars, and nonreducing sugars were higher than observed in seeds germinated for 48 h. On further germination up to 96 h, the contents of these sugars increased. Starch content, on the other hand, decreased. When the present observations are combined with those of a previous paper, it appears that germination of pulses for 24 h is a reasonably good treatment for reduction of flatus-producing carbohydrates as well as avoiding excess losses of the available carbohydrates.

Legumes, widely grown and consumed throughout the world, are excellent sources of proteins (20–40%) and carbohydrates (50–60%) and fairly good sources of thiamine, niacin, calcium, and iron (Aykroyd and Doughty, 1977). However, their wide acceptability is affected adversely due to the presence of flatulence factors as well as other antinutritional factors. Unavailable carbohydrates (15–25%) include substantial levels of oligosaccharides of the raffinose family of sugars (raffinose, stachyose, verbascose), which are well-known to produce flatulence in man and animals (Rackis, 1975; Olson et al., 1975; Reddy et al., 1980). Various processing methods have been tried to reduce the effect of these undesirable carbohydrates as well as to improve the digestibility of available carbohydrates in a variety of legumes (Iyengar and Kulkarni, 1977; Rao and Belavady, 1978; Reddy and Salunkhe, 1980; Reddy et al., 1980). In our previous paper (Jood et al., 1985), we reported the effect of common processing techniques on the contents of flatus-producing carbohydrates and recommended that germination for 24 h is the most suitable treatment for better utilization of legumes. However, all the methods described also affect the contents of available carbohydrates. Therefore, we report here the effect of those methods on the contents of available carbohydrates.

MATERIALS AND METHODS

Samples of five common legumes, Rajmah (*Phaseolus vulgaris*), Bengal gram (Cicer *arietinum*), black gram (*Phaseolus mungo*), red gram (*Cajanus cajan*), and broad bean (*Vicia faba*), were obtained from the Department of Plant Breeding, Haryana Agricultural University, Hissar, India.

Processing. The traditional methods of cooking legumes as described earlier (Jood et al., 1985) were followed in this investigation also. The samples were soaked in plain water and sodium bicarbonate solution (0.03%) for 6- and 12- h period at 25 °C. The samples thus soaked were cooked by boiling in water (4 times by weight) and autoclaved at 15 psi in double the amount of water. The soaked water was decanted before cooking. The samples to be germinated were surface sterilized with 1% sodium hypochlorite solution, washed thoroughly with distilled water, and placed at 30 °C on a damp filter paper and subjected to analysis at 24, 48, 72, and 96 h of germination. The 24-h germinated samples were shallow fried for 10 min in hydrogenated vegetable oil on a naked flame. The samples thus processed were dried at 80 °C in hot air oven until constant weight (48 h), ground in an electric grinder to pass through a 100-mesh sieve, and stored in an air-tight polyethylene bottle at room temperature (25 °C) until further analysis. The various antlytical procedures used were as follows:

The total water-soluble sugars were extracted according to the method of Cerning and Guilbot (1973). Starch was extracted from the sugar-free pellet by the method of Clegg (1956). Quantitative determination of total soluble sugars and starch was carried out according to the method of Yemm and Willis (1954). Reducing sugars were estimated by Somogyi's modified method (Nelson, 1944; Somogyi, 1945), and nonreducing sugars were estimated by calculating the difference between total soluble sugars and reducing sugars.

RESULTS AND DISCUSSION

Data included in Tables I–IV indicate that soaking of seeds for 6 h decreased the quantity of available carboh-

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