Comparative Study on the Lipoxygenase Activities of Some Soybean Cultivars

Judit Sz. Márczy,*,† Mária L. Simon,‡ Lajos Mózsik,§ and Béla Szajáni‡

Research Institute of Chemical Engineering, Hungarian Academy of Sciences, P.O. Box 125, Veszprém, Hungary H-8201, Department of Biochemistry, Attila József University, P.O. Box 533, Szeged, Hungary H-6701, and Cereal Research Institute, P.O. Box 391, Szeged, Hungary H-6701

The lipoxygenase levels of 10 soybean cultivars (McCall, Tresor, Recor, Essor, F054, Isz15, Chandor, Primor, Gadir, and Eszter) cultivated in Hungary in three consecutive years (1991, 1992, and 1993) were compared with regard to the activities and activity ratios of lipoxygenase isoenzymes. Surprisingly, both the activities and the activity ratios of the given cultivars were different in the three years studied. The differences in activity between the generations of a cultivar were found to be much larger than the differences between the different cultivars from the same year. It is suggested that the weather conditions play a considerable role in influencing the activities of the lipoxygenase isoenzymes in soybean cultivars.

Keywords: *Soybean lipoxygenase; lipoxygenase isoenzymes; synthesis; climatic effects*

INTRODUCTION

Lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.12) is an iron-containing dioxygenase that catalyzes the hydroperoxidation of polyunsaturated fatty acids and esters containing a *cis,cis*-1,4-pentadiene system (Hayaishi et al., 1975). The hydroperoxides formed are cleaved by hydroperoxide lyase, resulting in C_6 aldehydes in plants. These volatile carbonyl compounds have an objectionable odor, lending an undesirable flavor to food products. Hexanal is one such compound limiting the utilization of soybean in foods. Lipoxygenase and hydroperoxide lyase are considered to be predominantly responsible for 1-hexanal generation in processed soybean, and they have therefore been studied in detail (Matoba et al., 1985; Hildebrand et al., 1990; Cole, 1993; Moreira et al., 1993), with a view to decreasing hexanal production.

Lipoxygenase in soybean seeds is present in the form of three isoenzymes, i.e. Lox-1, Lox-2, and Lox-3, which differ in substrate specifity, optimum pH for catalytic activity, isoelectric point and thermal stability (Christopher et al., 1970; Axelrod et al., 1981; Siedow, 1991). They can be separated by ion-exchange chromatography (Christopher et al., 1970, 1972; Weber, 1974; Axelrod et al., 1981) or by isoelectric focusing (Funk et al., 1985). For the biocatalytic production of a natural aroma compound, lipoxygenase is needed on a large scale. Soybean flour is a very rich source of lipoxygenase isoenzymes. For our purposes, the alkaline isoenzyme (Lox-1) seems to be most suitable. Moreover, the presence of the neutral isoenzymes (Lox-2 and Lox-3) is disadvantageous since they catalyze the formation of an undesirable isomer product much more intensively than does Lox-1. In addition, Lox-2 and Lox-3 catalyze secondary reactions even under aerobic conditions, thereby reducing the yield of the desired product. The lipoxygenase isoenzyme activities in some soybean cultivars cultivated in Hungary were therefore screened and compared. The present paper reports on the results.

† Hungarian Academy of Sciences.
‡ Attila József University.
§ Cereal Research Institute.

MATERIALS AND METHODS

Soybean Cultivars. All of the soybean cultivars screened (i.e. McCall, Tresor, Recor, Essor, F054, Isz15, Chandor, Primor, Gadir, and Eszter) were from the Cereal Research Institute, Szeged, Hungary. These cultivars have been under observation for several years with regard to their yield and resistance to various environmental effects (e.g. drought resistance). Seeds were harvested in the crop years 1991, 1992, and 1993 at the station of the Institute located in Szeged, Hungary. During these three years, the cultivation was carried out the same way; fertilizer was not applied directly under the soybean, autumn wheat was applied as green crop, and the type of soil was black-earth. Seeds were bulked in separate lots and used for all experiments.

Apparatus. For the evaluations, a Biochrom 4060 spectrophotometer (Pharmacia, Uppsala, Sweden) was used.

Reagents. Linoleic acid and Tween 20 were purchased from Sigma Chemical Co., St.Louis, MO. All other chemicals were of analytical grade. For the preparation of sodium linoleate substrate, O_2-free water and an N_2 stream were used.

Preparation of Crude Extracts. Ten grams of soybean meal was defatted with 20 mL portions of petroleum ether at −5 to 0 °C and was then suspended and stirred with 7.5 volumes of 0.2 M sodium acetate buffer (pH 4.5) for 1 h at 2–4 °C. The suspension was filtered through cheesecloth and centrifuged (20000*g* for 40 min, at 4 °C). The pH of the supernatant was adjusted to 6.8. The crude extracts were stored frozen until use.

Lipoxygenase Assay. The method of Axelrod et al. (1981) was used with a slight modification. The activities of the lipoxygenase isoenzymes were determined via the increase in absorbance at 234 nm after addition of linoleic acid in 0.1 M sodium borate buffer (pH 9.0) for Lox-1 and in 0.2 M sodium phosphate buffer (pH 6.8) for Lox-2 and Lox-3. One unit is defined as the amount of enzyme producing 1 μmol of linoleic acid hydroperoxide/min.

Protein Content. Protein determinations were performed according to the method of Lowry et al. (1951), using BSA as standard.

Meteorological and Crop Development Data. Data relating to the crop years 1991, 1992, and 1993 were kindly provided by the Cereal Research Institute, Szeged, Hungary.

RESULTS AND DISCUSSION

For the biocatalytic production of a natural aroma compound, the alkaline isoenzyme of lipoxygenase is needed on a large scale. The neutral isoenzymes (Lox-2

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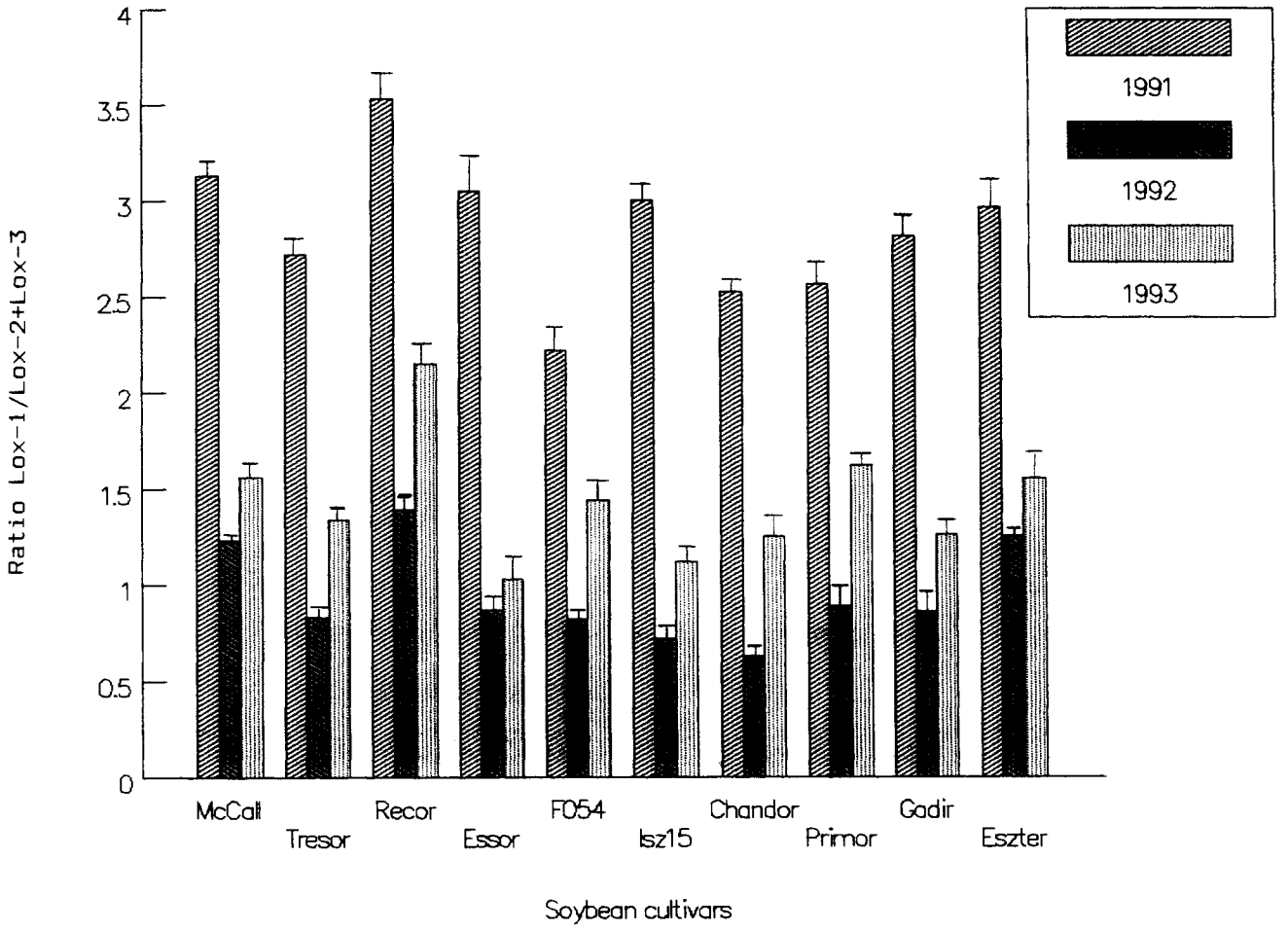

Figure 1. Comparison of lipoxygenase isoenzyme activity ratios for various soybean cultivars. The standard deviations of the samples were between 2.5 and 7.1% in all cases. In the experiments, 1 g of defatted soybean meal from each cultivar was processed.

and Lox-3) are disadvantageous because of undesired side-effects. An appropriate cultivar is needed as an available source of lipoxygenase. Therefore, we decided to examine cultivar differences and annual variations to select cultivars with high alkaline and low neutral isoenzyme activities. The lipoxygenase isoenzyme activities of 10 soybean cultivars cultivated in Hungary were therefore screened in three consecutive crop years, which differed appreciably with regard to the weather conditions (Table 1). The total lipoxygenase activities produced by any cultivar from 1991 were higher than those from 1992 (Table 2). At the isoenzyme level, the activities of Lox-1 were much higher than those of Lox-2 and Lox-3 in 1991, this being especially true for the cultivars McCall, Recor, and Essor. In 1992, the total activities of all isoenzymes were lower. The decreases were much larger for Lox-1 than for Lox-2 and Lox-3. In 1993, the trend of the activities was different with regard to the alkaline and neutral isoenzymes. The Lox-1 activities for all cultivars from 1993 were between the values from 1991 and 1992. For the neutral isoenzymes, the total activities were highest in 1993. On the other hand, the Lox-1 activities were higher than the activities of Lox-2 and Lox-3 in all cultivars from 1993, similar to the situation in 1991 (Table 2).

Data on seed yields are given in Table 3. These displayed a tendency similar to that of the enzyme activities. The seed yields were considerably lower in 1992 and 1993.

The biosynthesis of lipoxygenase isoenzymes in soybean is under genetic control (Hildebrand and Hymowitz, 1981; Kitamura, 1984). For this reason, it was supposed that the climate would not influence the proportions of the individual isoenzymes, and these proportions are characteristic for soybean cultivars. For comparative purposes, the ratio of the activities measured at pH 9.0 (Lox-1) and 6.8 (Lox-2 and Lox-3) was used.

In 1991, the activity ratios ranged from 2.22 (F054) to 3.53 (Recor), in 1992, from 0.63 (Chandor) to 1.39 (Recor), and in 1993, from 1.03 (Essor) to 2.15 (Recor). It is noteworthy that in all years Recor had the highest Lox-1/(Lox-2 + Lox-3) activity ratio (Figure 1). It is

Table 1. Meteorological Data Relating to the Main Developmental Stages of Soybean Growth in the Szeged Area

year	developmental stage[a]	total rainfall, mm	humidity, %	av temp, °C	no. of sunshine hours	no. of hot days (max temp >30 °C)
1991	I	139	69	15.8	440	6
	II	99	69	22.2	234	16
	III	67	73	20.0	260	6
	IV	305	70	19.3	934	28
1992	I	96	63	18.2	488	4
	II	16	63	21.5	301	10
	III	16	56	24.9	332	26
	IV	128	61	21.5	1121	40
1993	I	45	60	19.4	557	9
	II	56	57	21.7	293	13
	III	28	55	15.9	284	17
	IV	129	57	19.0	1134	39

[a] Developmental stages: I, from sowing to anthesis; II, period of flowering; III, from end of flowering to ripening; IV, from sowing to ripening (full vegetation period).

Table 2. Comparison of Total Activities of Lipoxygenase Isoenzymes in Various Soybean Cultivars[a]

	activity, units					
	Lox-1			Lox-2 + Lox-3		
cultivar	1991	1992	1993	1991	1992	1993
McCall	146 240	43 334	91 392	46 720	35 136	58 752
Tresor	85 094	22 618	69 120	31 334	27 379	50 492
Recor	146 490	40 960	111 600	41 510	29 491	51 708
Essor	144 860	26 111	43 500	47 520	29 952	42 108
F054	92 946	21 773	92 880	41 811	26 611	64 440
Isz15	122 688	18 432	71 796	40 896	25 728	64 356
Chandor	74 752	16 538	75 600	29 696	26 547	60 480
Primor	114 560	17 472	97 384	44 800	19 622	60 132
Gadir	129 894	23 846	86 784	46 234	27 648	68 124
Eszter	119 520	25 984	98 800	40 320	20 782	63 752

[a] The standard deviations of the samples were between 5.5 and 20.8% in all cases. For the comparison, 1 g of defatted soybean meal from each cultivar was processed.

Table 3. Comparison of Seed Yields of Various Soybean Cultivars

	yields, kg/acre		
cultivar	1991	1992	1993
McCall	976	641	517
Tresor	1128	414	495
Recor	1556	698	630
Essor	554	310	280
F054	1261	328	496
Isz15	1023	698	525
Chandor	1048	277	523
Primor	1418	622	681
Gadir	1186	442	542
Eszter	1213	501	676
$SD_{5\%}$	275	85	98

clear that the activity ratios of the given cultivars differed in the three years. The values from 1992 especially differed significantly from those relating to the other two years. It is suggested that the biosynthesis of lipoxygenase isoenzymes is sensitive to climatic factors, the sensitivities for the individual isoenzymes differing considerably.

Table 1 details the weather in the Szeged area during the main developmental stages of soybean growth in the three years in question. Rainfall is considered to be the most important factor influencing of ontogeny of soybean plants. In this respect the years 1992 and 1993 were similar; 1992 was more disadvantageous for soybean because of the extremely low amount of precipitation in the flowering and ripening periods. With regard to the number of hot days and the average temperature in the full vegetation period (developmental stage IV), 1992 and 1993 were again similar. It is interesting that during the ripening period in 1992 both the number of hot days and the average temperature were unusually high. We suggest that the hot summer and the drought in 1992 were responsible for the marked differences in both the total activities and the activity ratios of the lipoxygenase isoenzymes. These effects were less pronounced in 1993.

It appears from the results that the levels of the lipoxygenase isoenzymes are influenced to different degrees by both cultivar and climatic effects. The weather conditions can exert a greater effect than the cultivar effect. No data have been found in the literature on the relationship between biosynthesis of lipoxygenase isoenzymes and climatic factors. The present observation of such a relationship should be considered in studies involving soybean lipoxygenase isoenzymes.

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