J. Serb. Chem. Soc. 69 (8–9) 635–640 (2004) JSCS –3190 UDC 633.34+54–116:632.15 Short communication

# SHORT COMMUNICATION Variation in the chemical constituents of soybean due to industrial pollution

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# (Received 19 November 2003, revised 28 April 2004)

*Abstract*: The two varieties of soybean (*Soybean Bragg and Soybean JS-71-05*) were collected from an industrial site (IS) and from a non-industrial site (NIS) for the study of their chemical composition and fatty acids profiles by gas liquid chromatography (GLC). These studies revealed large changes in the major and minor fatty acids of the soybean seeds due to the effect of chemical pollutants. There was a significant decrease in the amounts of major and minor fatty acids, such as myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3), in the seeds from industrial site. The changes in the chemical composition due to chemical pollutants showed mixed results.

*Keywords*: pollutant, pollution, gas liquid chromatography, chemical analysis, instrumental analysis, macromolecules, lipids, fatty acids, soybean, industrial site, non-industrial site.

### INTRODUCTION

Soybean species are widely used as components of food in all areas of the world. The species are adaptable to varying conditions of soil and climate, proliferate profusely and are generally considered as shrubs. With growing industrialization, the macromolecular composition of plants has been subjected to changes due to chemical pollutants. The effect of chemicals and agrochemicals on plant macromolecules has attracted worldwide attention of researchers.<sup>1</sup> The significance and mechanism of such changes are still poorly defined. The few studies carried out so far indicate formation of free radicals which interact with proteins and lipids in the cell wall and membranes leading to their oxidation.<sup>2,3</sup>

Some researchers have studied the change in plant composition to assess the level of pollution.<sup>4–6</sup> A study of the effect of chemical pollutants on the molecular profile of plants from soybean seeds is of great significance due to the fact that the

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results may be indicative for other flora forms as well. Such studies have not yet been undertaken, so the results presented in the paper acquire added significance.

### EXPERIMENTAL

#### Sampling

The seeds of two soybean varieties (*Soybean Bragg & Soybean JS-71-05*) were collected within a 500 m radius of the Shaw Wallace Gelatin Company in Jabalpur (M.P.), India as representative of chemically polluted seeds. The other samples, representing pollution-free seeds were collected from areas remote far from this company. The chemical compositions of the soil near the Shaw Wallace factory and the pollution-free area were very similar.<sup>7</sup>

#### Methods of analysis

The chemical composition of the seeds was estimated by standard methods recommended by AOAC.<sup>8</sup> The moisture content was studied according to Pearson,<sup>9</sup> the crude fiber content as by Idems.<sup>10</sup> The ash content was detected as per Pearson.<sup>11</sup> The carbohydrate content was determined as per methods given by Nelson.<sup>12</sup> The protein content was determined by the microkjeldhal method as described by Sadasivan.<sup>13</sup> The calcium content method was reported by Piper<sup>14</sup> and the phosphorus content was determined as reported by Summer.<sup>15</sup>

#### Extraction of oil and GLC analysis

Oil was extracted from 100 g of soybean powder using methanol: chloroform (2:1 v/v) in a soxhlet apparatus. The chloroform – methanol layer after separation was concentrated. The residue was taken up in petroleum ether and allowed to settle. The petroleum ether layer was decanted and distilled off to obtain the oil. The obtained oil was subjected to saponification using 0.5 M alcoholic KOH at 65 °C and the fatty acid methyl esters were prepared by methanolysis of the fatty acid.<sup>16</sup> The fatty acid methyl esters were analyzed by gas liquid chromatography (Perkin Elmer 8700) with a flame ionization detector, using a DB-1 capillary column with nitrogen as the carrier gas (flow rate 20 ml/min). The oven temperature was 250 °C and the detector temperature 260 °C. Standard fatty acid methyl esters obtained from Sigma Chemicals (USA) were employed for identification. Three solvent blanks were also prepared and run.

### RESULTS AND DISCUSSION

Two varieties of soybean seeds (*Soybean Bragg* and *Soybean JS-71-05*) were studied for the lipid profiles and chemical constituents. The analyses were carried out on industrial site seeds and compared with non-industrial site seeds. Reports of such earlier studies<sup>5,6,17,18</sup> also indicated changes in the lipid profiles and chemical constituents due to chemical pollutants.

dustrial and medistrial site (per 100 g)											
Soybean varieties	Moisture	CFC	Total ash	Lipid	Carbohy- drate	Protein	NPN	Calcium	Phos- phorus		
Soybean Bragg (NIS)	9.46 ±0.38	3.28 ± 0.25	$\begin{array}{c} 3.70 \\ \pm \ 0.31 \end{array}$	19.51 ± 0.50	20.48 ± 0.66	36.39 ± 0.27	$\begin{array}{c} 0.36 \\ \pm \ 0.02 \end{array}$	0.16 ± 0.02	1.30 ± 0.22		
Soybean Bragg (IS)	9.67 ± 0.10	3.23 ± 0.03	4.43 ± 0.16**	$18.12 \pm 0.08^{**}$	$25.33 \pm 0.02^{***}$	$34.02 \pm 0.04^{***}$	$0.23 \pm 0.07^{***}$	$0.08 \pm 0.02^{***}$	$0.44 \pm 0.02^{***}$		

TABLE I. Chemical composition of seeds of Soybean Bragg and Soybean JS-71-05 from the non-industrial and industrial site (per 100 g)

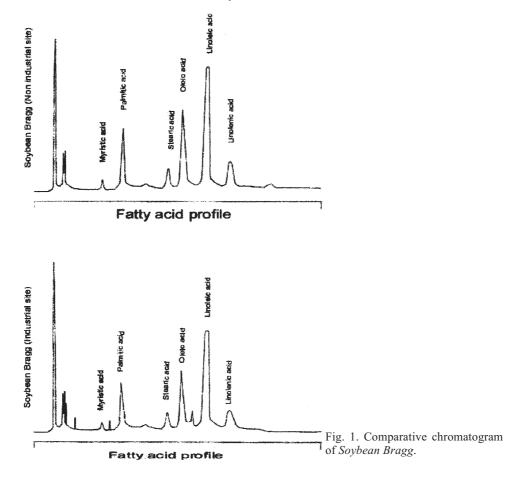
Soybean Moisture	CFC	Total ash	Lipid	Carbohy- drate	Protein	NPN	Calcium	Phos- phorus
Soybean 8.31 JS-71-05 $\pm 0.02$ (NIS)	2.85 ± 0.23	4.44 ± 0.04	18.80 ± 0.16	$\begin{array}{c} 20.60 \\ \pm \ 0.06 \end{array}$	37.17 ± 0.12	$\begin{array}{c} 0.52 \\ \pm \ 0.01 \end{array}$	0.22 ± 0.01	1.44 ± 0.02
$\frac{\begin{array}{c} \text{Soybean} \\ \text{JS-71-05} \\ (\text{IS}) \end{array}}{9.34} \pm 0.08^{***}$	$3.22 \pm 0.03^{*}$	5.88 ± 0.09***	$17.64 \pm 0.05^{**}$	$22.06 \pm 0.05^{***}$	$36.29 \pm 0.02^{***}$	$0.60 \pm 0.02^{***}$	$0.14 \pm 0.02^{***}$	$1.30 \pm 0.03^{***}$

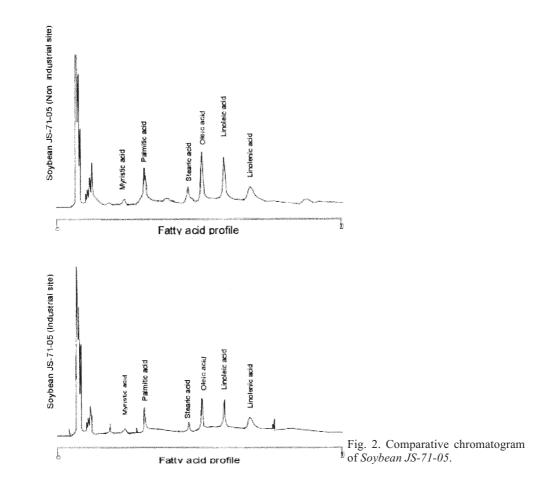
TABLE I. Continued

Mean ± SD (n = 4); \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001 significantly different from non-industrial site, respective soybean variety (Student t- test)

CFC : Crude fiber content, NPN : non-protein nitrogen

The results of this study showed that there were no significant changes in the moisture and crude fiber content of industrial site (IS) *Soybean Bragg*, but significant increases were observed in *Soybean JS-71-05*. The total ash content was in-





creased in the IS seeds of soybean varieties. The values of the lipid, total protein, calcium and phosphorus were significantly decreased in the IS seeds as compared to those of the non-industrial sites (NIS). Once however, the carbohydrate content was significantly increased in the IS seeds. A decreased amount of non-protein nitrogen was found in *Soybean Bragg* whereas an increased content was found in the IS seeds of *Soybean JS-71-05*. (Table I).

Instrumental analysis of the fatty acid profile for both species of IS and NIS gave specific patterns of GLC chromatograms.

The composition of the fatty acids in both IS *Soybean* varieties, *viz*. myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) showed a decrease in their respective quantities. The results are shown in the comparative GLC chromatograms (Figs. 1 and 2).

Typically, thermally powered industries, such as the Shaw Wallace Company, emit sulfur dioxide, carbon dioxide, carbon monoxide, methane and particulates. The composition of this soot may vary due to the source of the raw materials, the given processes and the atmospheric condition.

These pollutants enter the plants through cuticles/stomatal cavities and interact with biological components to produce ionic species or free radicals. These free radicals interact with proteins and lipids resulting in their oxidation and the liberation of more free radicals. This can lead not only to metabolic disorder but also to damage of DNA and RNA molecules. The fact that atmospheric pollutants may change the composition of lipid molecules was summarized by Heath.<sup>19</sup> Some airborne pollutants enter in the soil like polycyclic aromatic hydrocarbons, heavy metals, volatile and semi volatile solvents. These are absorbed by roots and they find their way into the metabolic cycles of these macromolecules.<sup>20–22</sup> The changes in plant molecules serve as indicators of the pollutants present in the environment.

Acknowledgements: We are thankful to the Indian Oil Corporation, New Delhi, for the GLC analysis of fatty acids and to Dr. K. K. Mishra, Head of the Chemistry Department, RDVV, Jabalpur (M.P.). The computer assistance by Mr. Pramod Kumar Srivastava is also acknowledged.

#### ИЗВОД

## ВАРИЈАЦИЈЕ У САДРЖАЈУ ХЕМИЈСКИХ КОМПОНЕНТИ СОЈЕ ЗБОГ ИНДУСТРИЈСКОГ ЗАГАЂЕЊА

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Два варијетета соје (Soybean Bragg и Soybean JS-71-05) прикупљена са индустријских и неиндустријских подручја проучена су са гледишта својих хемијских компоненти и садржаја масних киселина методом гасно-течне хроматографије. Проучавања су указала на велике промене садржаја главних и споредних масних киселина у зрнима соје под дејством хемијских загађивача. Запажено је значајно смањење садржаја важнијих и споредних масних киселина, као што су миристинска киселина (14:0), палмитинска киселина (16:0), стеаринска киселина (18:0), олеинска киселина (18:1), линолна киселина (18:2) и линолеинска киселина (18:3) у зрнима са индустријских подручја. Промене садржаја других хемијских компонената због хемијског загађења дала су мешовите резултате.

(Примљено 19. новембра, ревидирано 28. априла 2004)

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