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Effects of chelating agents on protein, oil, fatty acids, and minerals in soybean seed[#]

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ABSTRACT

Soybean seed is a major source of protein and oil for human diet. Since not much information is available on the effects of chelating agents on soybean seed composition constituents, the current study aimed to investigate the effects of various chelating agents on soybean [(*Glycine max* (L.) Merr.)] seed protein, oil, fatty acids, and mineral concentrations. Three chelating agent [citric acid (CA), disodium EDTA (DA), and salicylic acid (SA)] were applied separately or combined with ferrous (Fe^{2+}) ion (CA + Fe, EDTA + Fe, and SA + Fe) to three-week-old soybean plants. After application, the plants were allowed to grow until harvest maturity under greenhouse conditions. The results showed that CA, DA, SA, and Fe resulted in an increase of oleic acid from 13.0% to 33.5%. However, these treatments resulted in a decrease of linolenic acid from 17.8 to 31.0%. The treatments with CA and SA applications increased protein from 2.9% to 3.4%. The treatments DA + Fe and SA + Fe resulted in an increase in oil from 6.8% to 7.9%. Seed macro- and micro-elements were also altered. The results indicated that the CA, SA, DA, and Fe treatments can alter seed protein, oil, fatty acids, and mineral concentrations. Further studies are needed for conclusive results.

Keywords: Chelating Agents; Fatty Acids; Minerals; Oil; Protein; Soybean Seed Composition

1. INTRODUCTION

Soybean seeds are a major source of protein and oil [1], carbohydrates, minerals, and secondary metabolites such as lignin and isoflavones [2-5]. The quality of the soybean seed and the flavor and shelf-life of the oil produced are determined by protein, oil, and fatty acid content and quality. The five main fatty acids present in soybean seed are palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) [6]. The higher linoleic and linolenic acids were reported to lower the shelf-life of the oil due to oxidation of polyunsaturated acids [7,8]. Hence, the soybean industry prefers soybean with lower percentages of linoleic and linolenic acids, and higher percentage of oleic acid. Earlier reports have studied the growth response of plants in a nutrient solution containing chelating agents with ferric (Fe^{3+}) ions. In this experiment, soybeans were exposed to different dosages of metal ion and chelating agents. In the absence of iron (Fe) or when only the chelating agent was present, the plant showed chlorosis. Spraying iron sulfate ($FeSO_4$) solution on the plants resulted in plants' recovery from the chlorosis [9]. Iron movement in the growth medium is reported to be dependent on the chelating agents, pH, and exposure time to ligand [10]. In an experiment on monocotyledonous species, where NaFe-EDDHA chelate [(Fe ethylenediamine di (O-hydroxyphenylacetate)] was used, it was reported that the root system was better able to absorb ferrous Fe^{2+} ion than the ferric Fe^{3+} ion [11].

Although using synthetic chelating agents helped to understand plant nutrition, the micronutrient carrier from soil to plants [12] and the uptake of iron-chelating agents by plants are still not fully understood. Application of micronutrients is important for soybean yield and seed quality, especially in nutrient-deficient soil [13-21]. Iron is critical for chlorophyll formation, photosynthesis, respiration [21]. Most soils contain abundant levels of iron. However, at higher pH or in poor drainage, iron may not be available to plants, leading to iron deficiency [11,12, 14,15,18-20]. Combinations of chelating agents can increase iron availability [9,11,12], and thus may improve seed constituents. Studies have reported that the use of citric and oxalic acid on lemongrass resulted in better

[#]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

growth compared to the use of EDTA [22]. The objective of this study was to investigate the effects of applying chelating agents citric acid, salicylic acid, and the synthetic agent Disodium EDTA, alone and in combination with ferrous ion on soybean seed protein, oil, fatty acids, and minerals. To our knowledge, this is the first study to investigate the effects of chelating agents on seed protein, oil, and fatty acids in soybean.

Our hypothesis was that the plants may absorb the naturally occurring low binding (10^{3-16}) citric and Salicylic acids as chelating agents for metal ions over EDTA, which have higher binding constants (10^{10-25}) to different metal ions [23] for transporting iron and other micro- and macro-nutrients into the plants. Our hypothesis was based on the fact that metal ions (M) bind differently to different chelating agents (called also ligand, L), and form metal-ligand complexes (ML). The higher amount of ML than L or M in the solution implies that the ligand has a high binding constant with the metal M. Details on Stability Constants (log K_1) of various metal chelates that defines this complex chemistry are reported elsewhere [23].

2. MATERIALS AND METHODS

2.1. Treatment and Experimental Design

A greenhouse experiment was carried out at Mississippi Valley State University. Seeds of glyphosate-resistant Pioneer 95Y70 cultivar were planted in late April 2010, and seeds were collected at harvest maturity by early October 2010. Soil treatments were: Control, $FeCl_2$ (Fe), citric acid (CA), disodium EDTA (DA), salicylic acid (SA), CA + Fe, DA + Fe, and SA + Fe. Each set of treatment consisted of four pots. Four soybean seeds were planted in each pot. After three weeks of plant growth, the pots were treated with the appropriate chelating compound. To prepare the solution, 2.4 mmol of the chelating compound were dissolved in 2000 mL of deionized water (DI); then 250 mL of the solution were used per pot. This equates to 0.30 mmol of sample dissolved in 250 mL of water per pot. The chemical application was applied twice within a week at vegetative stage. The soil moisture levels of pots in the greenhouse were regularly monitored using soil water potential sensors. The pots were watered twice a week. The plants were grown under greenhouse conditions of natural light and temperature. Temperature during the soybean growing season ranged from 21˚C to 40˚C.

2.2. Harvesting

Soybean seeds were collected at harvest maturity (R8) for seed protein, oil and fatty acid, and mineral analysis.

2.3. Seed Protein, Oil, and Fatty Acid Analyses

Seeds from each soybean pot were sampled and analyzed for protein, oil and fatty acids using near-infrared (NIR) reflectance diode array feed analyzer (Perten, Spring Field, IL, USA) [24,25]. Calibration curves have been regularly updated for unique samples according to AOAC methods [26,27].

2.4. Seed Mineral and Soil Analyses

Elemental analysis of the seeds and soil samples was carried out by Soil, Water, Plant and Animal Tissue Analysis Laboratory, University of Georgia, Athens, GA, USA [28].

2.5. Experimental Design and Statistical Analyses

The experiment was a randomized complete block design. Four replicates were used. Analysis of variance was conducted using Proc GLM in SAS [29], with level of significance of $p \leq 0.05$.

3. RESULTS

3.1. Seed Protein, Oil, and Fatty Acids

The citric acid (CA) and salicylic acid (SA) applications increased seed protein percentage by 2.9% and 3.4%, respectively (**Table 1**). Conversely, disodium EDTA (DA) + Fe, and SA + Fe applications decreased the protein percentage by 4.1% and 4.4%, respectively. The DA + Fe, and SA + Fe applications increased oil by 7.1% and 8.2%, respectively. The Fe, CA, DA, and SA applications decreased seed palmitic acid from 8.7% to 16.7%, with SA having the greatest effect. The CA+ Fe, and DA + Fe applications increased palmitic acid by 8.7% and 10.3%, respectively. The application of CA, CA + Fe, DA + Fe, and SA + Fe increased stearic acid from 4.7% to 9.3%, with CA and SA + Fe applications having the greatest increase of 9.3%. The Fe, CA, DA, SA and SA + Fe applications increased oleic acid by 13.0% to 33.0%, with CA having the greatest effect. The CA + Fe and DA + Fe applications decreased oleic acid by 13.3% and 17.0%, respectively. The DA and SA applications increased linoleic acid by 4.0% and 4.6%, respectively. The SA + Fe application decreased linoleic by 3.1%. The Fe, CA, DA and SA applications decreased linolenic acid by 17.7% to 31.3%, with SA having the greatest effect.

In summary, the treatments with $FeCl_2$, CA, DA, SA, CA + $FeCl_2$, DA + $FeCl_2$, and SA + $FeCl_2$ have different effects on the soybean seed protein, oil, palmitic, stearic, linoleic, and linolenic acid %. Application of CA increased oleic acid by 33.0%, but decreased linolenic acid percentage by 17.7%. Similar observation for SA appli-

Table 1. Mean values of protein, oil, and fatty acid percentage (%) as affected by treatments: C = control, Fe = $FeCl_2$, CA = citric acid, DA = disodium EDTA, SA = salicylic acid, CA + Fe = Citric acid + $FeCl_2$, DA + Fe = Disodium EDTA + $FeCl_2$, and SA + Fe = salicylic acid + $FeCl_2$.

Treatments	Protein	Oil	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:2)
C	41.2cd	18.4cd	12.6b	4.3d	18.75c	54.4cb	9.6ab
Fe	41.4bcd	18.0cd	11.5c (–8.7%)	4.4d	21.3b (+13.0%)	55.8ab	7.1cd (–25.0%)
CA	42.4ab (+2.9%)	19.0bcd	10.8d (–14.3%)	4.7a (+9.3%)	25.0b (+33.0%)	53.2cd	7.9c (–17.7%)
DA	42.1abc	19.0bcd	11.0cd (–12.7%)	4.3d	21.4b (+13.8%)	56.6a (+4.0%)	6.9d (–28.1%)
SA	42.6a (+3.4%)	18.2d	10.5d (–16.7%)	4.4dc	21.7b (+15.4%)	56.9a (+4.6%)	6.6d (–31.3%)
CA + Fe	40.9d	18.3cd	13.7a (+8.7%)	4.5b (+4.7%)	16.3d (–13.3%)	55.8ab	9.8a
DA + Fe	39.5e (–4.1%)	19.7ab (+7.1%)	13.9a (+10.3%)	4.5bc (+4.7%)	15.6d (–17.0%)	55.5ab	10.5a
SA + Fe	39.4e (–4.4%)	19.9a (+8.2%)	12.5b	4.7a (+9.3%)	22.9b (22.8%)	52.7d (–3.1%)	8.9b

*Means given within a column with the same letter are not significantly different at $p \leq 0.05$. Four replicates were used. The numbers in the brackets with % are increase (+) or decrease (–) from the control for a given seed composition constituents.

cation where oleic acid increased by 15.4% and linolenic acid decreased by 31.3%. The CA and SA applications decreased palmitic acid by 14.3% and 16.7%, respectively. Applications of CA and SA increased protein percentage by 2.9% and 3.4%, respectively.

3.2. Ratios of A_1/A_2

Table 2 shows the effect of chemical applications on the unsaturated fatty acids linoleic and linolenic compared with palmitic, stearic, and oleic acids. That is, A_1 = linoleic + linolenic acids, and A_2 = palmitic, stearic, and oleic acids. For control, the ratio was 1.79. Higher ratio means higher % of linoleic and linolenic acids were produced. For Fe, CA, DA, SA, and SA + Fe applications, the ratios ranged from 1.51 to 1.73. Application of CA had the lowest ratio (1.51) followed by SA + Fe application (1.53). Lower ratios means lower % of linoleic and linoleic acids and higher percentage of oleic acid produced. Applications of $FeCl_2$, CA, SA, DA, and SA + $FeCl_2$ increased oleic acid.

3.3. Macro and Micro-Elements in Seed

Chemical treatments had varying degrees of influence on the composition of macro-elements in the seed. Application of $FeCl_2$ increased calcium by 25%, Mg by 7.0%, and N by 5.5% (**Table 3**). The CA application increased N by 6.4%, and decreased P by 10.3%. The SA application increased N by 7.4% and decreased S by 12.5%. The $FeCl_2$ application increased B by 16.0%, Cu by 7.0%, Fe by 14.0%, and Zn by 14.0%. The CA application increased B by 11.0%, Cu by 17.0%, Fe by 31.0%, Na by 30.0%, and Zn by 15.0%. The SA application increased B by 11.0%, Cu by 24.5% and decreased Fe by 10.4%. The DA application increased Cu by 50.0%, Mo by 57.0% and Zn by 28.0% (**Table 3**).

3.4. Macro- and Micro-Elements in Soil

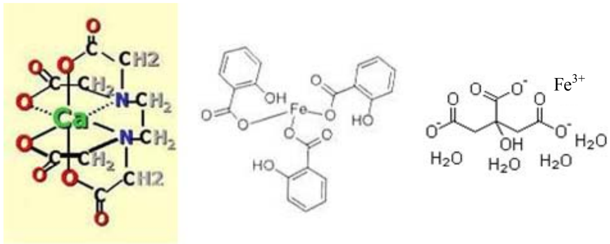
The percentage (%) of elements in soil were N = 2.11%, K = 0.35%, Ca = 3.3%, Mg = 0.21%, S = 0.17%, and P = 0.02% (**Table 4**). The micro-elements concentration (mg/kg) in soil were Mn = 84.0, Fe = 65.0, B = 0.6, Zn = 6.5, Na = 81.0, Cu = 0.5, and Mo = 0.1 (**Table 4**).

3.5. Stability Constants of Chelating Agents, CA, SA and DA

Table 5 shows the binding constants of chelating agents used in this study. A strong metal ions-EDTA complex is made, and this is due to the EDTA-binding constant, 10^{10-25}, which varies depending on the type of metal ions. Citric ($10^{2.8-11.85}$) and salicylic acids ($10^{2.7-16.35}$) weakly bind with the metal ions. Structures of EDTA, salicylic acid, and citric acid and their binding to metal ion are presented in **Figure 1**, and the chemical structure of fatty acids are shown in **Figure 2**. The chemical structures in **Figure 2** were drawn using a ChemSkecth program provided by a chemistry development software program (freeware) [30].

4. DISCUSSION

Research is scarce on the influence of the application of chelating agents to soil on soybean seed protein, oil, fatty acid, and mineral contents. In an earlier study on soybean plants grown in nutrient solution, it was reported that the chelating agent was absorbed by plants only when the chelating agent concentration was higher than the metal ion concentration [9]. It was also concluded that chelating agents applied in the absence of iron are absorbed by the plant and bound to the iron in the plant, causing chlorosis [9]. Spraying such plants with $FeSO_4$ resulted in rapid recovery and chlorosis elimination [9]. It was also reported that in nutrient solutions of chelating



EDTA SA CA
Binding constants:
10^10.7 10^6.5-11.85 10^11.85

Figure 1. Chemical structures of chelating agents and their binding constants [23].

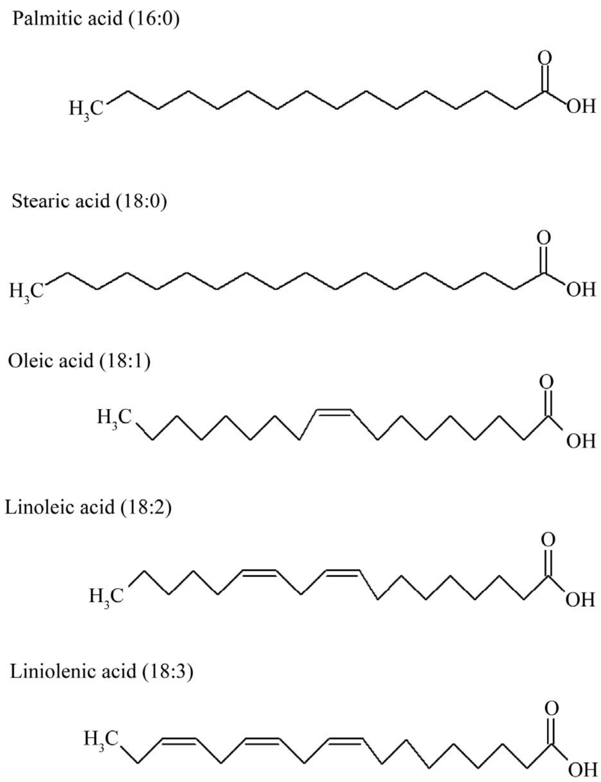


Figure 2. Structures of five fatty acids [30].

agent and ferric ion, the roots of kidney beans and soybeans seeds competed with the chelating agents for the ferric ion, and the competitive effect was overcome by adding more metal ion into the nutrient solution [31]. It was shown in radish sprouts grown in a growth medium that Ethylenediaminedisuccinic acid (EDDS) and hydroxy-iminodisuccinic acid (HIDS) produced the highest apparent mobility of iron from the bottom layer of the medium (initially 10^{-4} M Fe(III)) to the upper layer (no iron), followed by glutamatediacetic acid (GLDA), ethylenediaminetetraacetic acid (EDTA), implying that the HIDS is an effective carrier of metal ion from the solution than EDTA. The mobility of the ion depended upon the chelating agent, pH, and exposure time [10]. Their observation was that the plant may be converting ferric ion into ferrous before absorption [11]. Although using synthetic chelating agents may help us understand metal ion uptake by plants, their usefulness as a micronutrient carrier is still debatable [12].

In our study, ferrous ion applications altered the composition of fatty acids and minerals in seeds. For example, the chelating agents, especially CA and SA, had major effects on seed protein, fatty acids, and mineral composition. The treatments DA + Fe and SA + Fe resulted in an increase in oil content. Application of CA increased seed sodium by 30%, but manganese concentration did not change with any application. The lack of response of seed manganese to the application of chelating agents could be due to low binding constant of manganese with all the chelating agents [23]. However, we cannot explain the high level of manganese in leaves during the flowering stages [32]. In spite of the importance of macro- and micro-elements [13-21] for soybean growth and development, further research is needed to understand the mechanisms controlling the relationships between chelating agents and nutrient uptake by plants and the relationships between chelating agents and nutrient deposition in the seed. Altering the composition of soybean seed with applications of CA, SA,

Table 2. Ratios of A_1/A_2 for various treatments, where A_1 = Linoleic + Linolenic, A_2 = Palmitic + Stearic + Oleic, C = Control, Fe= $FeCl_2$, CA= Citric acid, DA= Disodium EDTA, SA= Salicylic acid, CA+ Fe= Citric acid + $FeCl_2$, DA + Fe= EDTA + $FeCl_2$, SA+ Fe= Salicylic acid + $FeCl_2$.

		A_1/A_2
C	= (54.4+9.58)/(12.55 + 4.30 + 18.75)	= 1.79
Fe	= (55.75 + 7.13)/(11.50+ 4.4+ 21.3)	= 1.69
CA	= (53.2+ 7.9)/(10.75 + 4.65 + 25.0)	= 1.51
DA	= (56.6+ 6.85)/(11.0 + 4.33 + 21.35)	= 1.73
SA	= (56.9+ 6.6)/(10.5 + 4.4 + 21.7)	= 1.74
CA + Fe	= (55.8 + 9.8)/(13.7 + 4.5 + 16.3)	= 1.90
DA + Fe	= (55.5 + 10.5)/(13.9 + 4.5 + 15.6)	= 1.94
SA + Fe	= (52.7+8.9)/(12.5+4.7+22.9)	= 1.53

Table 3. Macro-elements and trace (micro) elements in soybean as affected by chemical treatments, where, C = control, Fe = $FeCl_2$, CA = Citric acid, DA = Disodium EDTA, SA = Salicylic acid, CA + Fe = Citric acid + $FeCl_2$, DA + Fe = Disodium EDTA + $FeCl_2$, and SA + Fe = Salicylic acid + $FeCl_2$.

Treatments	Macro elements (%) Ca	K	Mg	N	P	S	
C	0.40cd	2.20bc	0.29c	5.80c	0.78ab	0.32a	
Fe	0.50a (+25.0%)	2.09bc	0.31a (7.0%)	6.12b (+5.5%)	0.78a	0.33a	
CA	0.41bcd	2.07c	0.30abc	6.17b (+6.4%)	0.70d (–10.3%)	0.32a	
DA	0.40cd	2.13bc	0.29c	6.39a (+10.0%)	0.76abc	0.300b	
SA	0.38d	2.25bc	0.28c	6.23ab (+7.4%)	0.73bcd	0.28c (–12.5%)	
CA + Fe	0.43bc	2.70a (+23.0%)	0.31ab (+7.0%)	6.15b (+6.0%)	0.81a	0.27c (–15.6%)	
DA + Fe	0.42bc	2.22 bc	0.29bc	5.79c	0.74bcd	0.30b (–6.0%)	
SA + Fe	0.38d	2.18bc	0.26d (–6.3%)	5.8c	0.72cd (–7.7%)	0.32a	
Treatments	Trace elements (mg/kg)						
	B	Cu	Fe	Mn	Mo	Na	Zn
C	38.20c	9.14e	82.43cd	63.23abc	11.54b	457.4bc	62.13c
Fe	44.40a (+16.0%)	9.79d (+7.0%)	93.98b (+14.0%)	64.44ab	13.95b	330.2c	70.95b (+14.0%)
CA	41.95b (+11.0%)	10.69c (+17.0%)	108.3a (+31.0%)	61.72bc	11.37b	594.4a (+30.0%)	71.44b (+15.0%)
DA	38.60c	13.69a (+50.0%)	81.13cd	67.2bc	18.16a (+57.0%)	493.0ab	79.9a (+28.0%)
SA	42.53b (+11.0%)	11.38b (+24.5%)	73.83e (–10.4%)	61.9bc	13.62b	389.0bc	63.18c
CA + Fe	42.31b (+11.0%)	11.34b (+24.0%)	77.93de	56.6c	13.06b	406.7bc	64.34c
DA + Fe	39.67c	10.01d (+9.6%)	86.35c	68.64a	13.62b	408.9bc	69.5b (12.0%)
SA + Fe	39.00c	10.31cd (+13.0%)	82.35cd	69.24a	17.33a (+50.0%)	366.7bc	68.83b (11.0%)

*Means given within a column with the same letter are not significantly different at $p \leq 0.05$. Four replicates were used. The numbers in the brackets with % are increase (+) or decrease (−) from the control for a given seed composition constituents.

Table 4. Chemical and physical characteristics of soil.

Soil	Chemical and physical properties of soil
Soil texture	Sandy clay
Base saturation	95.1
pH ($CaCl_2$)	5.70
Equivalent water pH	6.30
Organic matter (OM %)	36.9
Macro-elements (%)	
N	2.11
S	0.17
P	0.02
Ca	3.30
K	0.35
Mg	0.21
Trace elements (mg/kg)	
Cu	0.50
Fe	65.0
Mn	84.0
Mo	0.10
Zn	6.50
Na	81.0
B	0.60

Table 5. Stability constants of EDTA, citric acid, and salicylic acid with elements [23].

Chelating agent	Ca	Mg	Mn	Fe^{2+}	Fe^{3+}	Zn	Cu
EDTA	10.7	8.69	13.56	14.3	25.70	16.5	18.8
Citric acid	3.50	2.80	3.20	3.20	11.85	4.50	6.10
Salicylic acid	N/A*	4.70	2.70	6.55	16.35	6.85	10.6

*Not available.

and ferrous ion will further expand our knowledge about the role of chelating agents in seed constituents quality.

5. CONCLUSION

The application of CA and SA increased seed protein by 2.9% and 3.4%, decreased palmitic acid by 14.3% and 16.7%, decreased linolenic by 17.7% and 31.3%, and increased oleic acid by 33.0% and 15.4%, respectively for CA and SA. All seven treatments (**Table 1**) altered the seed composition of all macro- and micro-elements, except manganese. Further research is needed before conclusive observations are made.

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