Reduction of Pb(II) Ion in Soybean Seeds (*Glycine max*) Using Corncob Liquid Smoke

T Handayani¹, D Xyzquolyna¹, Y Pranoto², A Suratman³

¹Food Science and Technology Department, Faculty of Agriculture, Universitas Ichsan Gorontalo, 96123 Gorontalo, Indonesia
²Food Science and Technology Department, Faculty of Agricultural and Technology, Universitas Gadjah Mada, 55167 Yogyakarta, Indonesia
³Chemistry Department, Mathematic and Natural Science Faculty of Mathematic and Natural Sciences, Universitas Gadjah Mada, 55167 Yogyakarta, Indonesia

Email: trihandayani.kimia@gmail.com

Abstract. This research aims to study the ability of corncob liquid smoke for reduce of Pb(II) Ion in soybean seeds and the influence of its constituent compounds in reducing of Pb content in soybean seeds. The liquid smoke was obtained from corncob via pyrolysis, precipitation and two-time distillation. The process of reducing Pb content in soybean seeds is by soaking soybean seeds in liquid smoke with variations in the concentration of liquid smoke (0; 12.5; 25; 50 and 100%). Results show that the phenol, carbonyl and acid total content of corncob liquid smoke were 1.22%; 5.65%; and 9.60%; respectively. The corncob liquid smoke with concentration of 100% is able to reduce Pb content in soybean seeds by 48.93%. The components of phenol, carbonyl and acid in liquid smoke reduce after the chelation process. This shows that phenol, carbonyl and acid components influence the reduce Pb content in soybean seeds.

Keywords: Pb(II) Ion, Soybean Seeds, Corncob Liquid Smoke

1. Introduction

The effects of heavy metals on the environment and human health increasingly concern many parties. The entry of heavy metals into the ecosystem accumulates in plants and animals and may finally can potentially endanger to human health. One of the heavy metals that can enter and accumulate in plants is lead. Problems in hemoglobin synthesis, compromise of the kidneys, digestive tract, joints, and reproductive system, and results in acute or chronic damage to the nervous system can be caused by high exposure to Pb in humans[1].

Agricultural land contaminated by lead heavy metals will also produce contaminated food crops. In Indonesia, one product of food crops with a relatively high level of consumption is soybeans. The content of Pb metal in soybean seeds grown on intensification land was 0.63 ppm [2] This value exceeds the maximum limit of Pb contamination in soybean seeds according to SNI 7387-2009 which is 0.5 ppm [3]. In Fandong South China, the Pb metal content of soybean seeds was 0.34 ppm [4], in Argentina was 0.85 ppm [5], meanwhile, in Cordoba, Central Argentina was about 1.52 to 2.55 ppm [6]. And if the Pb contaminated soybeans are continuously consumed, the Pb metal will accumulate in
the human body so that it can endanger health. Therefore, it is necessary for soybean seeds to get pre-treatment to reduce the Pb level, so that the accumulation of Pb metal inside the human’s body can be avoided.

One of the methods for reducing heavy metal content is the chelation method. This method was performed by adding a chelating compound to bind heavy metals, forming complexes between heavy metals and chelating compound [7]. Hartati et al [8] reported that the Coconut shell liquid smoke can be used as a chelating agent to reduce Pb metal content in soybean seeds. Soaking soybean seeds for 2 hours using coconut shell liquid smoke can reduce Pb heavy metal content of whole soybean seeds by 63.41%.

Corncob is one of the agriculture wastes in Gorontalo Province, Indonesia which increases in number along with the increase of corn production [9]. Corncobs can be converted to liquid smoke through the pyrolysis process and used as bioflavor, color formers in smoke food products, food preservatives [10], organic insecticides [11], and wood preservatives [12] because liquid smoke reported contains phenols, acids, and carbonyl compounds. The utilization of corncob liquid smoke to reduce heavy metal levels in food commodities has not been widely reported. While, according to Volesky [13] the carboxyl, hydroxyl, and carbonyl functional groups are reported to possess high affinity in forming chelate complexes with heavy metal ions. Based on the principle of HSAB (Hard Soft Acids Bases) of Pearson[14], the benzene aromatic ring in phenol compound and carbonyl functional groups are classified as a soft base species. This causes the interaction of phenol and carbonyl groups with heavy metal ions, which are soft acids, that is preferred. This research aims to study the ability of corncob liquid smoke for reducing of Pb(II) Ion in soybean seeds and the influence of its constituent compounds in reducing Pb content in soybean seeds.

2. Materials and Method
The materials used in this research were hybrid corncobs waste of Pertiwi-2 variety with 90 days harvest age, soybean seeds obtained from Gorontalo traditional market, standard solution of 1000 ppm Pb(NO$_3$)$_2$, Cd(NO$_3$)$_2$, HClO$_4$, chloride acid, nitrate acid, folin ciocalteu reagent, acetic acid, phenol, and acetone (Merck, Germany).

2.1. Procedure to make corncob liquid smoke
Corn cobs are dried under the sun until the water content is less than 15%, followed by the analysis of cellulose, hemicellulose, and lignin. Corncobs were pyrolyzed at 400 °C, the resulting liquid smoke is precipitated for 24 h and then followed by two-time distillation at 98 °C [15]. Afterward, the redistilled liquid smoke of corncobs was analyzed for phenol and carbonyl content using spectrophotometer UV-Vis on wavelength 750 nm; acid content was analyzed with acid-base titration; and pH measured by pH meter.

2.2. Absorption of Pb(II) by soybean seeds
The standard solution of Pb(NO$_3$)$_2$ with the concentration of 2 ppm is added to 100 g of soybean seeds then soaked for 2 hours. Stirred with a speed scale of 600 rpm. Furthermore, the soybean seeds were dried in a cabinet dryer at 500 °C for 24 hours. The atomic absorption spectroscopy at the wavelength of 283.8 nm used to analysis Pb metal content in soybean seeds.

2.3. Reduction process of Pb content in soybean seeds by using corncob liquid smoke
The chelation method was performed to reduce Pb content in soybean seeds. 5 g of soybean seeds contaminated by Pb were soaked in 25 mL of corncob liquid smoke with various concentrations of 0; 12.5; 25; 50 and 100%. The mixture was then stirred using magnetic stirrer for 2 hours at 600 rpm. The soybean seeds were further drained and washed twice using 15 mL demineralized water each. Afterward, they were dried in a cabinet dryer at 50°C for 24 hours [16], [2]. The Pb metal content of the soybean seeds was then analyzed by using the AAS at 283.3 nm wavelength.
The measure of Total acid, phenol, and carbonyl content: Total acid content of liquid smoke was analysed by using the AOAC method [17]. Measure 1 mL of redistilled liquid smoke and add aquadest until the volume was 100 mL. Mixed until homogeneous, and titrate with 0.1 N NaOH using phenolphthalein 1 mL.

\[
\text{total acid content (\text{\%})} = \frac{V \times N \times BM \times F_{\text{dil}}}{\text{mg sample}} \times 100\% 
\]

Where:
- \(V\) = volume of NaOH (ml)
- \(N\) = normality concentration of NaOH (N)
- \(BM\) = mass molecule of acetic acid
- \(F_{\text{dil}}\) = dilution factor

Determination of total phenol content [18] with slight modification. As much as 1 mL of redistilled liquid smoke was dissolved into 100 mL of redistilled water, then 20 mL was taken from it to dissolved again into 100 mL (dilution factor = 500x). One mL from each final solution was put into the tube, added with 5 mL of Na\(_2\)CO\(_3\) 2% alkali, incubated for 10 min at room temperature, then added with 0.5 mL of Folin-Ciocalteu reagent and shaken using vortex before 30 min of incubation at room temperature. Absorbance was measured at 750 nm. The total phenol content of distilled liquid smoke was calculated using the previously obtained standard curve equation.

\[
\text{phenol content (\text{\%})} = \frac{A \times F_{\text{dil}}}{\text{mg sample}} \times 100\% 
\]

Where:
- \(A\) = phenol mass of the measured sample (mg)
- \(F_{\text{dil}}\) = dilution factor

Determination of total carbonyl content [18]. As many as 5 mL of redistilled liquid smoke was diluted until the volume reaches 100 mL, then 5 mL was taken and diluted again until the volume reaches 100 mL (dilution factor 400x). The solution as a result of dilution was taken 1 ml and mixed with 1 ml 2,4-dinitrophenylhydrazine and 50 µL of conc. HCl. Heated at the 50 °C for 30 minutes. Cooled and added 8 mL KOH 1 N. Absorption reading was made at 480 nm by a spectrophotometer. The Beer-Lambert law holds only approximately for pure acetone at this wavelength.

\[
\text{carbonyl content (\text{\%})} = \frac{A \times F_{\text{dil}}}{\text{mg sample}} \times 100\% 
\]

Where:
- \(A\) = carbonyl mass of the measured sample (mg)
- \(F_{\text{dil}}\) = dilution factor

3. Results and Discussion

3.1. The water content, cellulose, hemicelluloses, and lignin content of corncob

The water content, cellulose, hemicelluloses, and lignin content of corncob was performed to determine the physicochemical properties of raw materials. In this study, the water content, cellulose, hemicelluloses, and lignin content of the corncob can be observed in Table 1.

| Water content (%) | Cellulose (%) | Hemicelluloses (%) | Lignin (%) |
|------------------|---------------|--------------------|------------|
| 7.46             | 35.14         | 37.31              | 6.98       |
The water content of the pyrolyzed material is no more than 15% [19]. Water levels that are too high will inhibit the combustion process, thereby reducing the liquid produced. Some of the heat energy that should be used to decompose the fiber will be used to evaporate the water contained in the material. The other research reported corncobs contain 22.27% of cellulose, 28.30% of hemicelluloses, and 19.95% of lignin [20].

3.2. The phenol, carbonyl, and total acid content of corncob liquid smoke
The content of phenol, carbonyl, and total acid from the liquid smoke by UV-Vis spectrophotometry can be observed in Table 2. They were 1.22%, 5.65%, and 9.60%, respectively.

| Phenol content (%) | Carbonyl content (%) | Acid total (%) | pH |
|-------------------|----------------------|---------------|----|
| 1.22              | 5.65                 | 9.60          | 2.05 |

The results obtained are lower than the phenol, carbonyl, and total acid content of the redistillation result of coconut shell liquid smoke reported by Darmadji [21], i.e. 3.90%, 7.10%, and 9.60%, respectively. This lower yield can be affected by the presence of moisture, pyrolysis temperature, or the amount of oxygen in the smoke generator [22].

3.3. The reduction of Pb levels in soybean seeds by means of corncob liquid smoke
The reduction of Pb content in soybean seeds by means of the corncob liquid smoke can be observed in Table 3. The concentration of liquid smoke affects its chelating ability. Liquid smoke with 100% concentration (without dilution) gives the greatest effect on reducing Pb metal content which is from 7.91 ppm to 4.04 ppm, or equal to 48.93%. The greater the concentration of the corncob liquid smoke, the more functional groups of carboxyl, hydroxyl and carbonyl in the liquid smoke can bind to the Pb metal.

| Liquid smoke concentration (%) | Pb (ppm) | % reduce |
|-------------------------------|----------|----------|
| Initial Pb content            | 7.91     | 0        |
| 0 (control)                   | 7.53     | 4.80     |
| 12.5                          | 6.76     | 14.54    |
| 25                            | 6.22     | 21.36    |
| 50                            | 5.28     | 33.24    |
| 100                           | 4.04     | 48.93    |

*Significantly different (α = 0.05)

The reduction of Pb level by the corncob liquid smoke can also occur due to the low pH of liquid smoke (Table 4). Speciation of Pb can be found predominantly at pH < 7 [23]. One-way ANOVA revealed there is any effect on the concentration of liquid smoke with a decrease of Pb (F_{test} > F_{table}; 424.277 > 3.11). Subsequent analysis by Duncan method indicated a significant difference in the decrease Pb levels between 0; 12.5; 25; 50 and 100% concentration of liquid smoke.

| Liquid smoke concentration (%) | pH | Final |
|-------------------------------|----|-------|
| Initial                       | 2.28 | 408   |
| 25                            | 2.25 | 3.70  |
| 50                            | 2.17 | 3.34  |
| 100                           | 2.04 | 3.05  |
3.4. The changes of the phenol content in corncob liquid smoke after chelation process

The chelation method in the process of reducing Pb content in soybean seeds was done by soaking soybean seeds for 2 hours while stirring. Phenol levels of liquid smoke reduced after the chelation process in soybean seeds (Table 5). This reduces in levels indicates that the components of liquid smoke phenol compounds have reacted with Pb ions in soybean seeds. Based on the principle of HSAB (Hard Soft Acid Base) from Pearson [7], aromatic rings in phenol compounds are soft base species. So the interaction with medium acidic Pb metal ions is probably influenced by covalent strength. In addition, aromatic rings in phenol compounds can increase the stability of the complex with Pb metal ions due to the resonance effect of the chelate ring [14]. The highest reduction in liquid smoke phenol levels after the chelation process occurred at the concentration of liquid smoke 25%, which amounted to 40.74%.

| Liquid smoke concentration (%) | Phenol content (%) |
|-------------------------------|-------------------|
| 12.5                          | 0.09              |
| 25                            | 0.27              |
| 50                            | 0.70              |
| 100                           | 1.22              |

3.5. The changes of the carbonyl content in corncob liquid smoke after chelation process

The liquid smoke carbonyl content of corn cobs that have been used for soaking soybean seeds has reduced (Table 6). The biggest reduce in carbonyl content occurred in the liquid smoke of corn cobs with a concentration of 25% that is equal to 17.47%. The carbonyl group in liquid smoke is a soft base species [7], so the interaction with Pb metal ions (medium acid) may be influenced by covalent strength. Reducing the level of liquid smoke carbonyl after the chelation process also shows that the carbonyl group in liquid smoke has reacted with Pb to form a Pb-carbonyl complex.

| Liquid smoke concentration (%) | Carbonyl content (%) |
|-------------------------------|----------------------|
| 12.5                          | 0.98                 |
| 25                            | 1.66                 |
| 50                            | 3.04                 |
| 100                           | 5.65                 |

3.6. The changes of the acid total content in corncob liquid smoke after chelation process

The total acid level in liquid smoke reduced after being interacted with Soybean seeds (Table 7). The largest reduction occurred in the liquid smoke of corn cobs with a concentration of 12.5%, which amounted to 18.26%. In the chelation process, the carboxylic group (-COOH) is deprotonated into a carboxylic ion (-COO-), the ion will chelate Pb, resulting in electrostatic bonds (due to charge differences) namely Pb\(^{2+}\) and 2 carboxyl ions (-COO-) [16]. This reduces in value indicates that the acid compound as one of the main components of the liquid cob corn constituent has reacted with Pb to form a Pb-carboxylic complex.

| Liquid smoke concentration (%) | Acid total content (%) |
|-------------------------------|------------------------|
| Initial                       | Final                  |

---

Table 5. The changes of phenol content in corncob liquid smoke after chelation process

Table 6. The changes of carbonyl content in corncob liquid smoke after chelation process

Table 7. The changes of acid total content in corncob liquid smoke after chelation process
4. Conclusion

Corn cob liquid smoke observed contains phenol, carbonyl, and acid components were 1.22%; 5.65%; and 9.60%; respectively. The corn cob liquid smoke with a concentration of 100% is able to reduce Pb content in soybean seeds by 48.93%. The components of phenol, carbonyl and acid in liquid smoke reduce after the chelation process. This shows that phenol, carbonyl, and acid components influence the reduce Pb content in soybean seeds.

Acknowledgment

The authors would like to thank the Ministry of Research, Technology and Higher Education for the funding as well as the research members and students who have contributed to this study.

References

[1] L. Seeul-Ji, L. Myoung-Eun, C. Jae Woo, P. Jin Hee, H. Keun Young, and J. Gee-Il, “Immobilization of Lead from Pb-Contaminated Soil Amended with Peat Moss”, J. of Chemistry, 2013.
[2] O. Andriyanto, Pengikatan Timbal (Pb) dan kadmium (cd) pada Biji Kedelai (Glycine max) Menggunakan Asam Asetat dan Asap Cair, Skripsi Fakultas Teknologi Pertanian, UGM, 2012.
[3] Badan Standar Nasional, SNI 7387-2009: Batas Maksimum Cemaran Logam Berat dalam Pangan, BSN, 2009.
[4] Z. Ping, L. Zhi-An, Z. Bi, Z. Han-Pin, and W. Gang, “Heavy Metal Contamination in Soil and Soybean near the Dabaoshan Mine, South China,” J. Pedosphere, 23(3), pp. 298–304, 2013.
[5] P. B. Tchounwou, C. G. Yedjou, A. K. Patlolla, and D. J. Sutton, Heavy Metal Toxicity and the Environment. SpringerLink, 2012.
[6] R. Lavado, C. Porcelli, and L. Alvarez, “Nutrient and Heavy Metal Concentration and Distribution in Corn, Soybean and Wheat as Affected by Different Tillage System in the Argentine Pampas,” J. Soil Tillage Res., 62, pp. 55–60, 2001.
[7] J. Basset, R. Denney, G. Jeffery, and J. Mendham, Vogel’s Textbook Of Quantitative Inorganic Analysis Including Elementary Instrumental Analysis, 4th ed. London: Longman Group UK, 1991.
[8] S. Hartati, P. Darmadjie, and Y. Pranoto, “Utilization of Coconut Shell Liquid Smoke to Reduce Lead (Pb) Levels in Soybean Seeds (Glycine max),” Agritech, 35(3), 2015.
[9] Badan Pusat Statistik, Produksi Padi, Jagung dan Kedelai Provinsi Gorontalo (Angka Ramalan II 2014, Badan Pusat Statistik Provinsi Gorontalo, 2014.
[10] J. Lingbeck, P. Cordero, C. O’Bryan, M. Johnson, S. Ricke, and P. Crandall, “Functionality of liquid smoke as an all-natural antimicrobial in food preservation,” Meat Sci, 92(2), pp. 197–206, 2014.
[11] R. S. Santos, “Characterization of Liquid Smoke from Coconut Shell as a Natural Pesticide for Hexamitodera Semivelutinia Beetle on Clove Trees,” Int. J. Appl. Chem., 12 (3), pp. 389–397, 2016.
[12] Rosalina, T. Tedja, E. Riani, and S. Sugianti, “An Environmental Friendly Pesticide From Bintaro (Cerbera odollam Gaertn) Liquid Smoke for Pine Wood Preservation Against A Subterranean Termite Coptotermes curvignathus Holmgren Attack,” Rasayan J Chem, 9(3), pp. 438–443, 2016.
[13] B. Voelsky, “Biosorption and me.,” Water Res, vol. 41, no. 18, pp. 4017–4029, 2007.
[14] R. Pearson, “Hard and Soft Acids and Bases HSAB Part I,” J Chem Ed, vol. 45, no. 9, 1968.
[15] Kuntjahjawati and D. P, “Identifikasi komponen volatil asap cair daun tembakau (Nicotiana tabacum L.) rajangan,” Agritech, 24 (1), pp. 17–22, 2004.

[16] Q. Li, L. Chai, Q. Wang, Z. Yang, H. Yan, and Y. Wang, “Fast Esterification of Spent Grain for Enhanced Heavy Metal Ions Adsorption,” Bioresour. Technol., 101, pp. 3796–3799, 2010.

[17] Anonim., Association of Official Analytical Chemistry: Official Methods of Analysis, 23rd ed, Washington DC, Benyamin Franklin, 1995.

[18] Senter S., Robertson J. and Meredith F., Phenolic Compound of The Mesocarp of Cresthaven Peaches During Storage and Ripening. J. Food. Sci., 54,1259-1268, 1989.

[19] Lappin G. and Clark L., Colorimetric Methods for determination of Traces Carbonyl Compound, Anal Chem., 23, 541-542, 1951.

[20] H. Zhang, R. Xiao, H. Huang, and G. Xiao, “Comparison of Non-Catalytic and Catalytic Fast Pyrolysis of Corncoeb in Fluidized Bed Reactor,” J. Bioresour. Technol., 100 (3), pp. 1428–1434, 2009.

[21] P. Darmadji, “Optimation of Liquid Smoke Purification by Redistilation Method,” Jurnal Teknol dan Ind. Pangan, 13 (3), pp. 267–271, 2002.

[22] M. Guillen, M. Manzanos, and L. Zabala, “Study of acommercial liquid smoke flavouring by means of gas chromatography-mass spectrometry and fourier transform infrared spectroscopy,” J. Agric. Food Chem., 43, pp. 463–468, 1995.

[23] M. Aroua, S. P. Leong, L. Teo, C. Yin, and W. M. A. Daud, “Real-Time Determination of Kinetics of Adsorption of Lead (II) onto Palm Shell-Based Actived carbon using Ion Selective Electrode,” Elsevier, 99(13), pp. 5766–5792, 2008.