Characterization of corncob liquid smoke and its application as chelating agent Pb ion in soybean seeds (*Glycine max*)

Handayani Tri[1*], Xyzquolyna Deyvie[1], Pranoto Yudi[2] and Suratman Adhitasari[3]
1. Department of Food Science and Technology, Universitas Ichsan Gorontalo, 96123 Gorontalo, INDONESIA
2. Department of Food Science, Technology Agriculture Faculty, Universitas Gadjah Mada, 55167 Yogyakarta, INDONESIA
3. Department of Chemistry, Mathematics and Natural Science Faculty, Universitas Gadjah Mada, 55167 Yogyakarta, INDONESIA
*trihandayani.kimia@gmail.com

Abstract
This study aims to characterize liquid smoke of corncob, to evaluate the ability of corncob liquid smoke as chelating agent Pb and the influence of its constituent compounds in reducing Pb ion content in soybean seeds. The liquid smoke was obtained from corncob via pyrolysis, precipitation and two-time distillation. The corncob liquid smoke was characterized by GC-MS to determine the component compounds in liquid smoke and UV-Vis spectrophotometry to determine the total phenol and carbonyl contents.

The research consisted of the concentration variation on corncob liquid smoke (0; 12.5; 25; 50 and 100%). The results show that liquid smoke by GC-MS detected 11 compounds. The total acid, phenol and carbonyl contents of corncob liquid smoke are 9.60%, 1.22% and 5.65% respectively. Compared to the other concentrations, the 100% concentration of corncob liquid smoke (without dilution) shows the best Pb reducing level of 48.93%. Phenol compound gives the highest reducing level of Pb content of the constituent compounds which is 48.29%, followed by the acetic acid of 46.14%, and acetone of 42.35%.

Keywords: corncob, liquid smoke, Pb ion, soybean seeds, chelating agent.

Introduction

The absorption of heavy metals from contaminated soil through the plant roots and the direct deposition of heavy metals from the atmosphere to the plant surfaces are the two main causes of heavy metal accumulation in food crops[1]. One of the heavy metals that is harmful to human health is lead (Pb). The accumulation of Pb metal inside the human's body may cause disruption of the nerve system, urinary system, reproductive system, endocrine system and heart[2].

Agricultural plants polluted by the heavy metal of Pb will produce contaminated food crops as well. Soybean is one of the food crops that is often consumed by people. However, it is reported to have the considerable capability in accumulating heavy metals. The Pb metal content of soybean seeds in Fandong South China was 0.34 ppm[1], in Argentina, was 0.85 ppm[3], meanwhile in Cordoba, Central Argentina was about 1.52 to 2.55 ppm. In Indonesia, the content of Pb metal in soybean seeds grown on intensification land was 0.63 ppm[4]. This value exceeds the maximum limit of Pb contamination in soybean seeds according to SNI 7387-2009 which is 0.5 ppm[5]. Therefore, it is necessary for soybean seeds to get pre-treatment in order to reduce the Pb level, so that the accumulation of Pb metal inside the human's body can be avoided.

Several techniques have been applied to remove heavy metal such as ion exchange, solvent extraction, ultra-filtration, adsorption, coagulation[6] and extraction using chelating agent[7]. Extraction using a chelating agent is also called chelation method. This method was performed by adding a chelating compound to bind heavy metal forming complexes between heavy metals and chelating compound[8]. The liquid smoke reported can be used as a chelating agent of Pb ions[4,9,10]. The liquid smoke from coconut shell can to reduce Pb content of soybean seeds by 63.41%[9].

Corncob is one of the agriculture wastes in Gorontalo which increases in number along with the increase of corn production[11]. Corncob liquid smoke of pyrolysis reported that it contains phenols, acids and carbonyl compounds. Therefore it was widely used as bio flavor, color formers in smoke food products, food preservatives[12], organic insecticides[13] and wood preservatives[14]. However, the utilization of corncob liquid smoke as a chelating agent of heavy metal has not been widely reported. In the meantime, according to Volesky[15], the carboxyl, hydroxyl and carbonyl functional groups are reported to possess high affinity in forming chelate complexes with heavy metal ions.

Furthermore, based on the principle of HSAB (Hard Soft Acids Bases), the benzene aromatic ring in phenol compound is classified as a soft base species[16]. This causes the interaction of phenol with heavy metal ions, which are soft acids, that is preferred. This study aims to characterize the liquid smoke of corncob, to evaluate the ability of corncob liquid smoke as a chelating agent and to evaluate the influence of its constituent compounds in reducing of Pb content in soybean seeds.

Material and Methods

The materials used in this research were hybrid corncobs waste of Pertiwi-2 variety with 90 days reaping time soybean seeds obtained from Gorontalo, a standard solution of 1000 ppm $Pb(NO_3)_2$, HCl, HNO_3, $HClO_4$, folin ciocalteu reagent, acetic acid, phenol and acetone (Merck, Germany). This research applied a completely randomized design with three replications. The data obtained were analyzed by using ANOVA followed by the BNT test ($\alpha = 0.05$).

Procedure to make corncob liquid smoke: Firstly, corncobs were dried, followed by the analysis of moisture content, cellulose, hemicellulose and lignin. Corncobs were pyrolyzed at 400 °C, precipitated for 24 hours and then followed by two-time distillation at 98 °C[17]. Afterwards, the redistilled liquid smoke of corncobs was analyzed for compounds content using GC-MS; total acid, phenol content and carbonyl content were analyzed by using the spectrophotometry UV-Vis on wavelength 750 nm. pH value was measured by using pH meter.

Absorption of Pb by soybean seeds: One hundred grams of dried soybean seeds were soaked in standard solution of $Pb(NO_3)_2$ with the concentration of 2 ppm for 2 hours. It was stirred with the speed scale of 600 rpm. Furthermore, the soybean seeds were dried in a cabinet dryer at 500 °C for 24 hours. The Pb metal content of soybean seeds was analyzed by atomic absorption spectroscopy at the wavelength of 283.8 nm.

The reduction process of Pb content in soybean seeds by using corncob liquid smoke: The reduction process of Pb content in soybean seeds was performed by the chelation method. 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. Afterwards, they were dried in a cabinet dryer at 50°C for 24 hours[4,18].

The Pb metal content of the soybean seeds was then analyzed by using the AAS at 283.3 nm wavelength. Moreover, the soybean seeds were soaked into the acetic acid solution, phenol and acetone. This step was performed to observe the effect of each constituent compound of the corncob liquid smoke (acetic acid, phenol and acetone) on the reduction of Pb content in soybean seeds.

Total acid content: Total acid content of liquid smoke was analyzed by using the AOAC method[19]. Measure 1 mL of redistilled liquid smoke and add aquades until the volume was 100mL. Mixed until homogeneous and titrated with 0.1*N* NaOH using phenolphthalein 1 mL.

Total phenol content: 1 mL of redistilled liquid smoke was dissolved into 100 mL of redistilled water, then 20 mL was taken from it to dissolve again into 100 mL (dilution factor = 500x). One milliliter from each final solution was put into the tube, added 5 mL of Na_2CO_3 2% alkali, incubated for 10 min at room temperature, then added 0.5 mL of Folin-Ciocalteu reagent and shaked 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[20].

Procedure to measure total carbonil content: 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-dinitrophenyl hydrazine and 50 µL of conc. HCl. Heated at the 50 ^{0}C for 30 minutes. Cooled and added 8 mL KOH 1 N. Absorption reading was made at 480 nm by spectrophotometer. The Beer-Lambert law holds only approximately for pure acetone at this wavelength.[21]

Results and Discussion

The characteristics of corncob raw material (water content, cellulose, hemicelluloses and lignin content): The characterization of raw materials was performed to determine the physicochemical properties of raw materials. In this study, the water content, cellulose, hemicelluloses and lignin content of the corncob were 7.46%, 35.14%, 37.31% and 6.98% respectively. The water content of the pyrolyzed material is 10% to 15%[22]. Excessive water content can inhibit the combustion process and reduce the distillate of the liquid smoke. The corncobs contain 22.27% of cellulose, 28.30% of hemicelluloses and 19.95% of lignin[23]. The fiber content in the cobs is influenced by corn varieties, length of planting and planting conditions.

The analysis result of compounds content of liquid smoke by GC-MS and UV-Vis spectrophotometry: The analysis result of corncob liquid smoke by GC-MS observed that the samples analyzed contain 11 peak indicating that 11 compounds are possible in the redistilled liquid smoke (Figure 1.). By using mass spectrometry, the compound can be clearly seen in the structure and the molecular weight (Table 1). Meanwhile, the contents of phenol, carbonyl and total acid from the liquid smoke by UV-Vis spectrophotometry were 1.22%, 5.65% and 9.60% respectively. The results obtained are lower than the phenol, carbonyl and total acid content of the re-distillation result of coconut shell liquid smoke i.e. 3.90%, 7.10% and 9.60% respectively[24]. This lower yield can be affected by the presence of moisture, pyrolysis temperature, or the amount of oxygen in the smoke generator[25].

The reduction of Pb content 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 figure 2. 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. The functional groups of acid compound, phenol and carbonyl can form complex compound with metals[26]. The carboxyl group on the acid and hydroxyl group on phenol can be deprotonated and will form bond with Pb metal[27].

The reduction of Pb level by the corncob liquid smoke can also occur due to the low pH of liquid smoke (Figure 3). Speciation of Pb^{2+} can be found predominantly at pH < 7[28].

The changes in the phenol content of corncob liquid smoke after interaction with soybean seeds: Figure 4 shows that the phenol content for each concentration variation of liquid smoke reduces after the soybean seeds were soaked. It indicates that the phenol compound in the liquid smoke has reacted with the Pb metal in the soybean seeds. Based on the principle of HSAB (Hard Soft Acids Bases) of Pearson[16], the benzene aromatic ring of the phenol compound is a soft base species. Therefore, its interaction with heavy metal ions that are soft acid, is preferred. The highest reduction of phenol content occurred in the corncob liquid smoke with 25% of the concentration which is 40,74%.

The changes of the acid content of corncob liquid smoke after interaction with soybean seeds: The acid content of corncob liquid smoke reduces after interaction with soybean seeds (Figure 5). This reveals that acid compounds contribute to reducing the Pb content of soybean seeds. The biggest reduction of 18.26% occurs in the corncob liquid smoke with a concentration of 12.5%. The carboxyl group (COOH) of deprotonated acids will form COO^- ions which can be bonded with a positive charge of the Pb ion[10]. A complex from bonding of Pb and acid compounds then will be formed.

The changes of carbonyl content of corncob liquid smoke after interaction with soybean seeds: The carbonyl content of the corncob liquid smoke that has been used for the soaking of soybean seeds were reduced as shown in figure 6. The highest reduction of 17.47% occurs in corncob liquid smoke with 25% concentration. This indicates that the carbonyl group in the liquid smoke has reacted with Pb ion to form a complex compound.

The reduction of Pb content of soybean seeds by means of phenol, acetone and acetic acid: The soaking of soybean seeds in phenol, acetic acid and acetone was performed in order to observe the effect of constituent compounds of corncob liquid smoke (phenol, acid and carbonyl) on reducing Pb content in soybean seeds. figure 7 shows that the phenol compound with the concentration of 1.22% is able to reduce the Pb metal content in soybeans from 7.91 ppm to 4.09 ppm, or equal to 48.29%. Acetone with a concentration of 5.65% is able to reduce the Pb content on soybeans from 7.91 ppm to 4.56, or equal to 42.35%. For acetic acid, the concentration of 9.60% is able to reduce the Pb content from 7,91 ppm to 4,26 ppm, or equal to 46.14%. Phenol gives the highest effect of Pb content reduction of 48.29%, followed by acetic acid with 46.14% and acetone which is 42.35%. These results show that the phenol compound, acetone and acetic acid possess different binding capabilities towards Pb ions.

Table 1
Mass spectrum of redistilled liquid smoke of corncob

Peak	tR (minute)	% area	Molecular weight/Molecular formula	The compound
1	2.016	3.32	62/ $C_2H_6O_2$	1,2-ethanediol
2	2.175	1.1	58/ C_3H_6O	Acetone
3	3.138	69.46	60/ $C_2H_4O_2$	Acetic acid
4	3.476	4.02	74/ $C_3H_6O_2$	2-propanone, 1-hydroxy
5	3.725	6.58	74/ $C_3H_6O_2$	Propionic acid
6	3.899	3.88	74/ $C_3H_6O_2$	Propionic acid
7	5.227	1.74	88/ $C_4H_8O_2$	1-hydroxy-2-butanone
8	6.888	5.07	96/ $C_5H_4O_2$	2-furancarboxaldehyde
9	7.125	0.57	102/ $C_5H_{10}O_2$	1-hydroxy-2-pentanone
10	12.635	1.84	94/ C_6H_6O	Phenol
11	16.348	2.42	124/ $C_7H_8O_2$	Mequinol

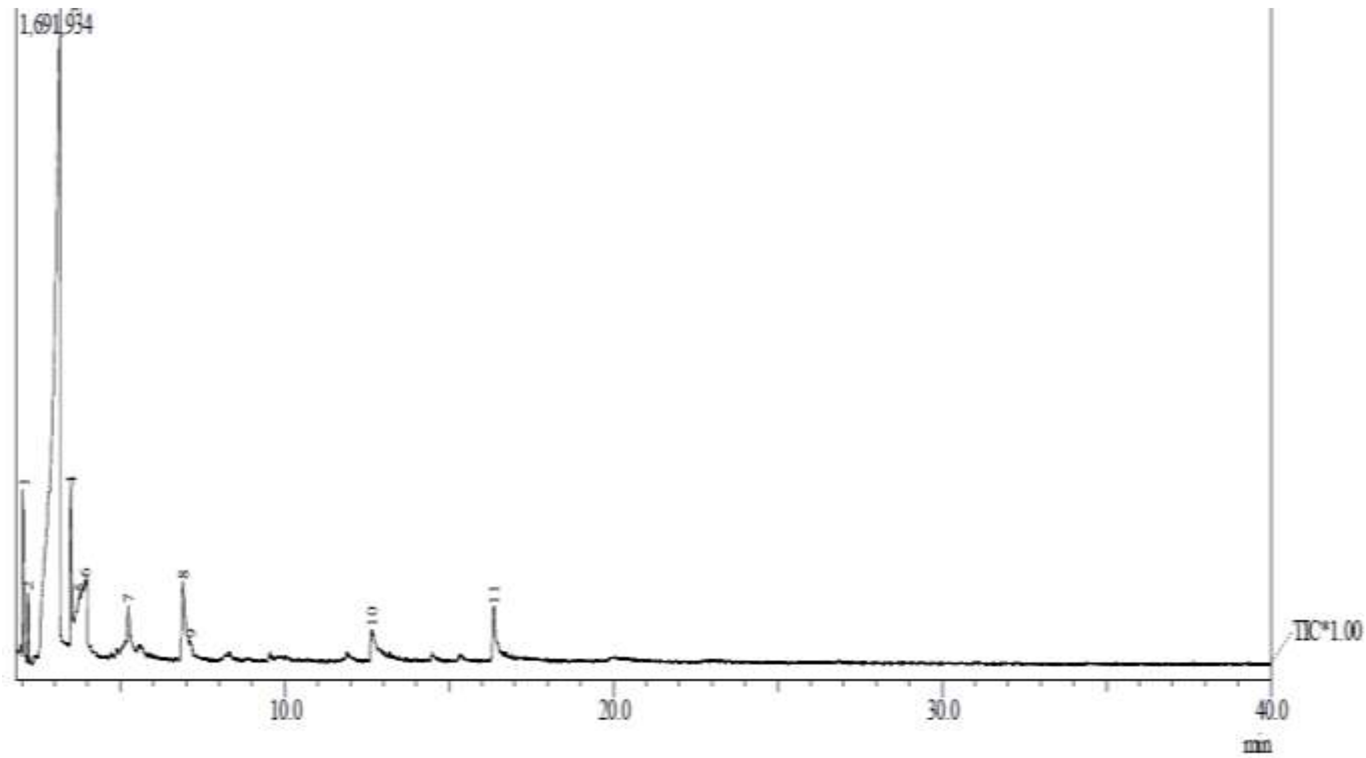


Figure 1: The chromatogram of corncob liquid smoke

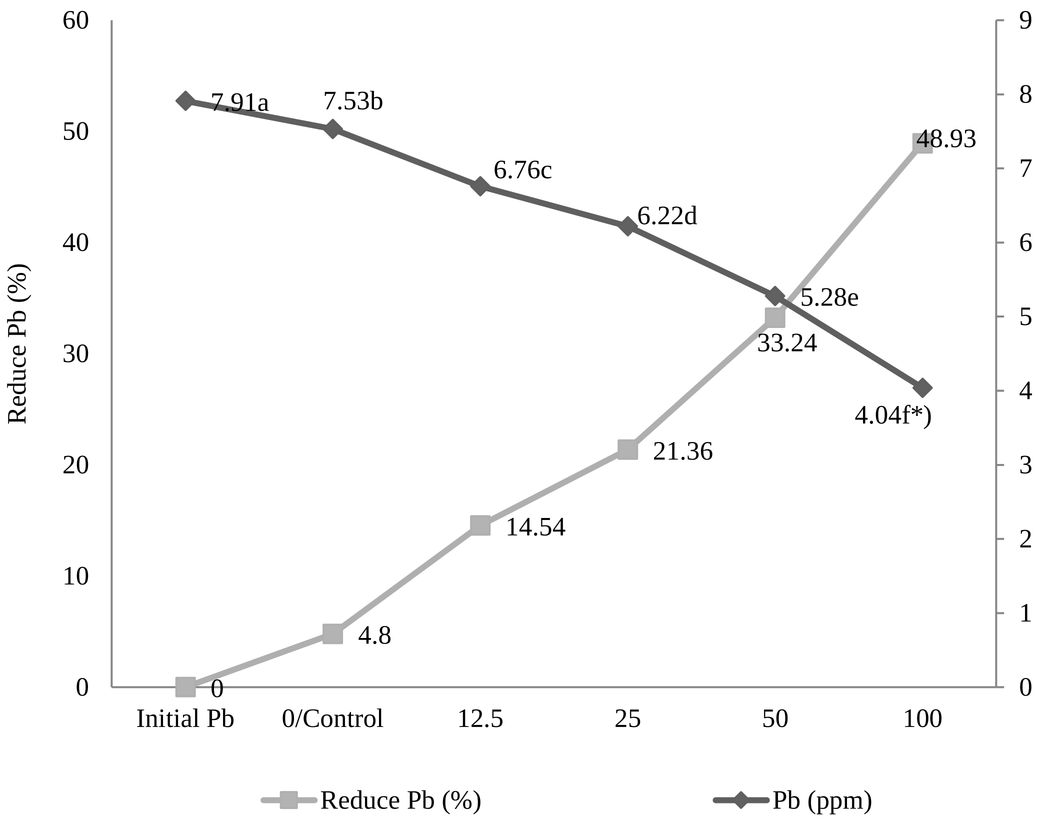
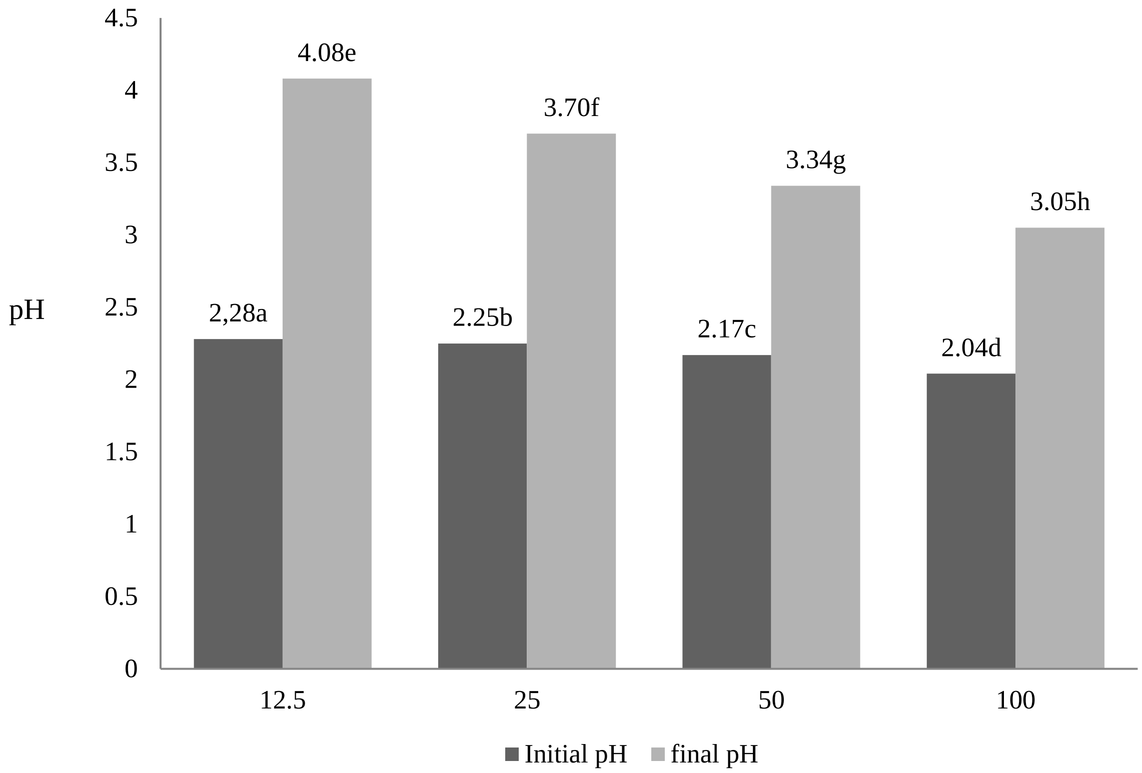
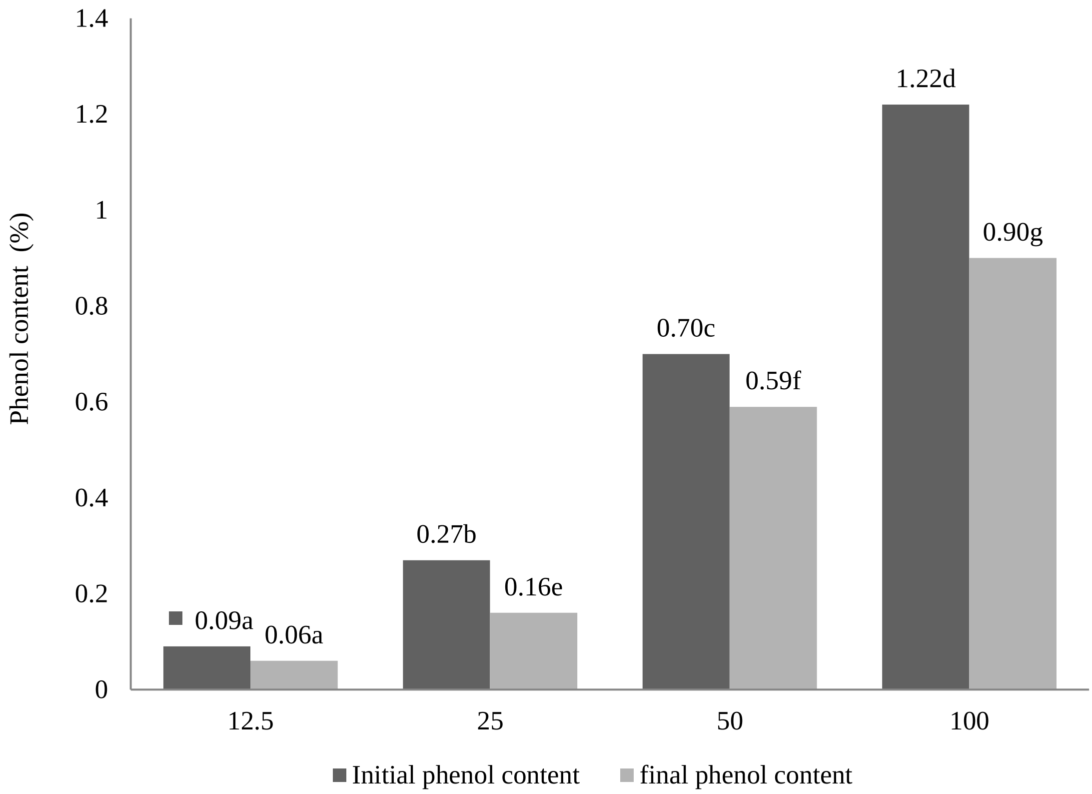


Figure 2: The reduce of Pb content in soybean seeds by corncob liquid smoke



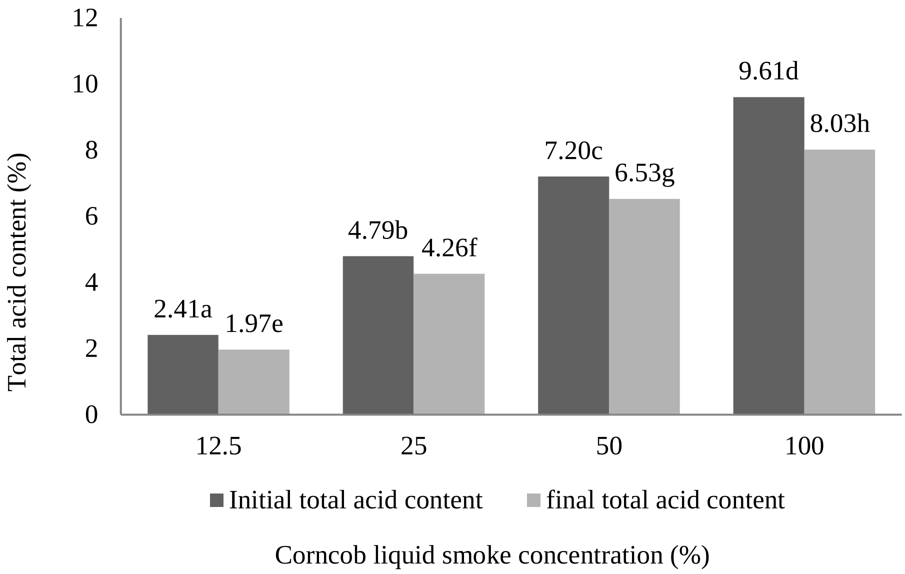
Corncob liquid smoke concentration (%)
*) different codes show significant difference result at 0.05 α level

Figure 3: The changes of pH of corncob liquid smoke after interaction with soybean seeds



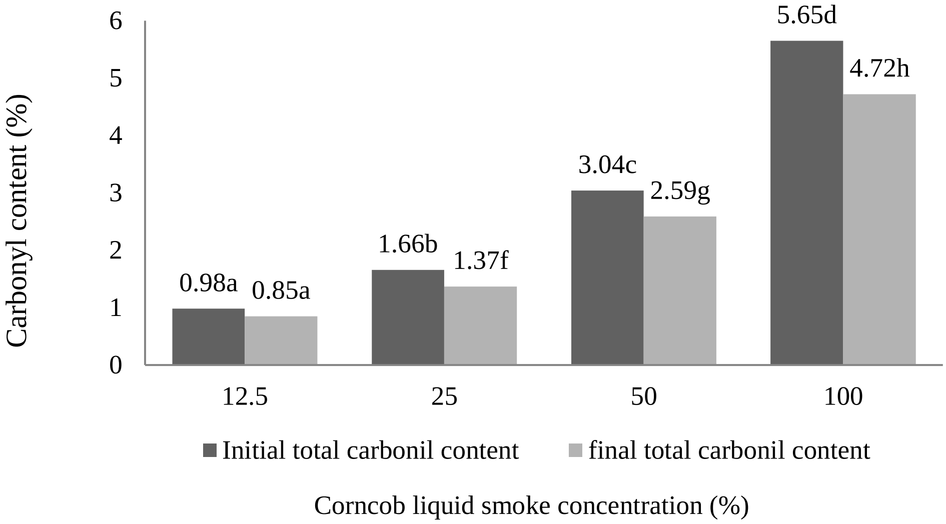
Corncob liquid smoke concentration (%)
*) different codes show significant difference result at 0.05 α level

Figure 4: The changes of phenol content of corncob liquid smoke after interaction with soybean seeds



*) different codes show significant difference result at 0.05 α level

Figure 5: The changes of acid content of corncob liquid smoke after interaction with soybean seeds



*) different codes show significant difference result at 0.05 α level

Figure 6: The changes of carbonyl content of corncob liquid smoke after interaction with soybean seeds

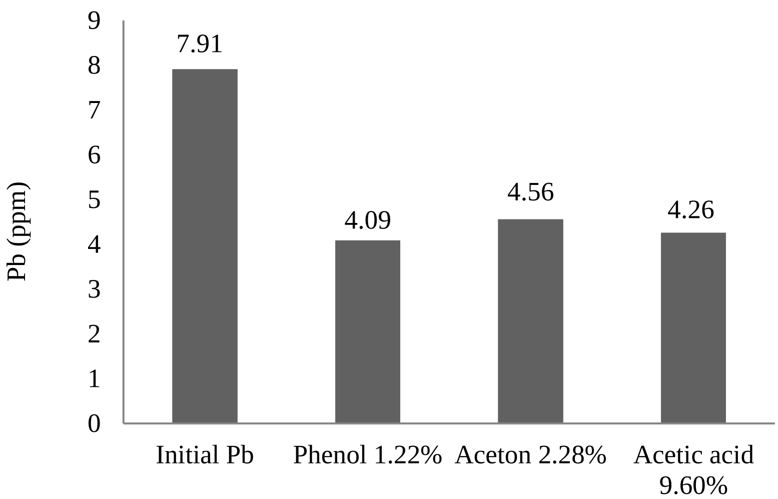


Figure 7: The reduce of Pb content of soybean seed by phenol, acetone and acetic acid solution

Conclusion

The corncob liquid smoke observed contains 11 compounds in the redistilled liquid smoke by GC-MS. The total acid, phenol and carbonyl contents of corncob liquid smoke are 9.60%, 1.22% and 5.65% respectively. The corncob liquid smoke can be used as Pb metal chelating agent in soybean seeds. The greater the concentration of liquid smoke, the greater the reduction of Pb content in soybean seeds.

The corncob liquid smoke with a concentration of 100% is able to reduce Pb content in soybean seeds by 48.93%. The content of phenol, acid and carbonyl compounds in liquid smoke contributes to the reduction of Pb content in soybean seeds. Phenol compound gives the biggest effect on Pb content reduction which is 48.29%, followed by the acetic acid of 46.14% and acetone of 42.35%.

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