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# Extraction and characterization of humic acid from plantation's solid organic waste composts

*Ekstraksi dan karakterisasi asam humat dari kompos limbah padat organik perkebunan*

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## *Ringkasan*

*Kompos dari limbah padat organik (LPO) perkebunan memiliki kandungan asam humat yang relatif tinggi. Namun, metode ekstraksi dan karakteristik asam humat asal kompos tersebut belum banyak diteliti. Oleh karena itu suatu rangkaian penelitian dilakukan dengan tujuan memperoleh paket teknologi ekstraksi dan menetapkan karakteristik asam humat asal kompos tandan kosong kelapa sawit (TKKS), kulit buah kakao (KBK), dan sisa pangkasan teh (SPT). Pengomposan dilakukan melalui tahapan pengumpulan limbah organik padat perkebunan, pencacahan, pencampuran dengan bioaktivator, inkubasi dan pemanenan. Hasil penelitian menunjukkan bahwa metode ekstraksi konvensional dengan larutan NaOH dalam atmosfer udara dapat digunakan untuk ekstraksi skala semi pilot. Jumlah asam humat yang dihasilkan dari kompos asal TKKS dan SPT lebih banyak apabila dibandingkan dengan asam humat asal kompos KBK. Waktu inkubasi pengomposan dan metode ekstraksi dengan gas $N_2$ atau udara yang digunakan tidak berpengaruh nyata terhadap perolehan asam humat. Pemurnian asam humat asal ketiga jenis LPO perkebunan dengan menggunakan kolom Sephadex G-50 mengindikasikan bahwa asam humat asal kompos TKKS, KBK, dan SPT tersebut memiliki fraksi bobot molekul rendah serta didominasi oleh asam amino aspartat dan glutamat. Konsentrasi asam amino dan senyawa karboksilat tertinggi terdeteksi pada asam humat asal kompos SPT.*

## Summary

The plantation's solid organic waste (SOW) composts contain relatively high humic acid (HA) substances. However, there is little information on extraction and characteristics of HA from the SOW-originated composts. An investigation has been conducted to determine extraction and characterisation of HA from empty fruit bunches of oil palm (EFBOP), cocoa pod husks (CPH), and tea cutting residues (TCR). Composting was conducted using the method that involved SOW collection, shredding, mixing with bioactivator, incubation, and harvesting. The results showed that conventional extraction method using NaOH solution under air atmosphere could be used for pilot scale extraction of humic acids (Has). Amount of humic acid from EFBOP and TCR were higher than that of CPH. The composting period and the extraction method under air or $N_2$ gas were not significantly affected the amount of the humid acid obtained. Purification of HA extracted from EFBOP, TCR, and CPH composts by using Sephadex G-50 column indicated that EFBOP, TCR, and CPH contained HAs with lower molecular weight fractions and predominated by aspartic and glutamic acids. The highest concentration of amino acids and carboxyl compounds were detected in the TCR-originated compost.

[***Key words***: Humic acid, solid organic-waste, biodecomposition]

## Introduction

HAs appear to be complex macromolecules of aromatic units with linked amino acids, peptides, amino sugars, aliphatic acids and other organic constituents (Stevensons, 1982). Several methods are available for the extraction and isolation of HA. Extraction procedures and extracting reagents of HA have been developed based on the principles where humic materials are soluble in dilute base. Gel permeation chromatography techniques indicated that extracts in dipolar aprotic solvents were predominantly of intermediate and low molecular-weight compounds. Sodium hydroxide solution, despite its oxidation effects, was the best solvent tested for the extraction which were representative for a wide range of soil humic substances (Hayes *et al*., 1975).

Extraction of humic acid by NaOH solution under $N_2$ gas would be replaced by the same solvent under air to meet condition of extraction. It is obvious that if the use of $N_2$ can be deleted, not only the speed of extraction will be increased, but the procedure can also be simplified considerably. No evidences were found indicating differences in amount of HA's attributed to NaOH extraction under air and $N_2$ gas as well as in contents of C, H,O, N and S (Tan *et al*., 1991). Tan & Giddens (1972) reported that elution of humic acid through Sephadex G-50 resulted only into two fractions: a high (>30,000) and a low (<15,000) molecular weight fractions. The main elements of humic acid are carbon (50-60%), oxygen (30-35%), hydrogen (4-6%), nitrogen (2-6%), and sulphur (0-2%) (Schnitzer, 1982). Whereas Tan (1998) reported that HA is usually rich in carbon (41-57%) and oxygen (33-46%) but lower in nitrogen contents (2-5%). Humic acid from fractions of soil organic matter are highly reactive polymeric compounds of various molecular weights, predominantly containing C, O, H, and N. The reactivity of the HA was attributed to the quality and quantity of the functional groups, especially oxygen (Challa *et al*., 1985).

HA has practical applications in agricultural practices, either through the conventional or biotechnological approach (Chen & Aviad, 1990; Tan *et al.*, 1991 ). Documentation have been established worldwide varying from characterization of HA isolated from different materials (Deiana *et al*., 1990; Lobartini *et al*., 1991&1992, Tan *et al*., 1992; and Goenadi 1997), oxide adsorption (Schulthess & Huang, 1991; Fontess *et al*., 1992), soft-weed herbicide (Goenadi, 1995) as well as its potential use as organic N sources for callus development (Goenadi & Mariska, 1995). HAs are less susceptible to microbial degradation than low molecular weight acids and represent a potential means by which a more permanent amelioration of Al toxicity may be effected (Harper *et al* ., 1995). Moreover, humic substances are used widely in modern agriculture as humic fertilizer that has been produced on industrial scale (Garcia *et al*., 1993). The commercial HAs commonly taken from lignite or leonardite, coal, peat, and other geologic deposits that inconsistent, limited and unrenewable. In contrast, EFBOP, CPH, and TCR are abundant and renewable sources of solid organic matter for HA production. Compost of solid organic waste (SOW) of plantations are assummed to contain relatively high concentration of HA that means it has more economical and environmentally friendly.

The aims of this research were to develop extraction method and to obtain physico–chemical characteristics of HA isolated from composts originating from solid organic wastes of plantation.

## Materials and Methods

### *Extraction and purification of humic acids from SOWs plantation*.

The plantation's SOW materials were obtained from Kertajaya, Rajamandala, and Montaya estates, West Java. The SOWs materials included empty fruit bunches of oil palm (EFBOP), cocoa pod husks (CPH), and tea cutting residues (TCR), respectively. Experiment were carried out at Ciomas Experimental Garden for composting and Analytical Laboratory of Biotechnology Research Unit for Estate Crop, Bogor for physico-chemical analyses. Selected incubation periods have been applied to the decomposition of EFOB, CPH, and TCR treated by *OrgaDec* bioactivator (*Organic*

*Decomposer*, Patent ID 0 000264S) (Goenadi *et al*., 2000). Composting procedures have followed to those reported ealier by Goenadi (1997). In brief, composting steps involved SOW collection, shredding, mixing with bioactivator, incubation, and harvesting. Shredding was conducted on-site, whereas composting was carried out at Ciomas Experimental Garden, Bogor. Dosages of bioactivator were 0.5% (w/w) for EFBOP and CPH, whereas that for TCR was 1.25% (w/w). Biodecomposition was allowed to take place for 28 days.

Experiment was arranged in a completely random design with 3x5 factorial patterns with three replicates. The first factor was the types of SOWs, whereas the second one was levels of incubation periods (0,7,14,21 and 28 d). HAs were extracted from plantation's SOW composts by three times of 100 mL of 0.5 N NaOH solution. The alkaline extracts were then fractionated into HAs by acidification with 2 N HCl to pH 2.0 (Schnitzer, 1982). According to the optimum incubation period of EFBOP, CPH, TCR, HA extraction was repeated by the same solvent under $N_2$ gas. Purification of HAs was conducted by elution through a chromatographic column (10x20 mm Phamacia) filled with Shepadex G-50 (Tan, 1998) using auto sampler-fraction collector LKB 7000. Elution pressure was assured by peristaltic pump (913 Mity Flex) at constant flow rate of 3 mL per minute. The E4:E6 ratio was determined according to the procedure described by Tan (1998). The slope of the spectral curve to identify molecular weight of HA was expressed as ratio of the absorbance at 465 and 665 nm (E4:E6).

### *Characterization of HAs extracted from plantation's SOWs*

A number of chemical characteristics of HAs were determined at Energy Resource Laboratory, Serpong. Carbon, hydrogen, and nitrogen were measured with the CHN microanalyzer (Leco USA CHN 1000) and oxygen was calculated as residual percentage (100-[C+H+N]) %. The sulphur element was determined by the method as described by Tan *et al*. (1991). Types and concentrations of amino acids were analyzed at Integrated Laboratory of Bogor Agricultural University by HPLC equipped with C18 column (Shimadzu).

Characterization of macromolecular structure of HAs can be conducted in several ways, such as infrared spectrophotometry. FTIR analyses were performed for identification of functional groups of HAs in the extracts. Infrared (IR) spectra were recorded from 4000 to 1000 $cm^{-1}$ in KBr pellets (1 mg of sample + 400 mg of dry KBr and press in a suitable die under vacuum at a pressure of 7,500 $kg/cm^{-2}$ for 20 min) (Schnitzer, 1982), with a FTIR Bio Rad 175C spectrometer.

## Results and Discussion

### *Extraction and purification of humic acid from plantation's SOWs.*

Humic acid concentration extracted by conventional method under air varies according to types of plantation's SOWs. In EFBOP, humic acid concentration increased with increasing incubation period up to two weeks 164.3 $g.kg^{-1}$ organic matter (OM) but at the third and fourth weeks, the contents were decreased (139.0 and 110.3 $g.kg^{-1}$ OM, respectively). Similar results were obtained from CPH experiment in which, the HA concentration at two weeks was about 110.0 $g.kg^{-1}$ OM. However, TCR compost contained increasing HA concentration with increasing period of incubation. The highest HA concentration of TCR occurred at fourth weeks incubation (129.8 $g.kg^{-1}$ OM). These results obtained from extracts of EFBOP, CPH, and TCR compost by conventional extracting method under air. Statistically, there is no significant effect of incubation period on HA concentration. However, the HAs concentration of EFBOP, CPH, and TCR composts were slightly much lower than that of humic acid extracted from composted separated manure (CSM) (184.0 $g.kg^{-1}$ OM) that have been reported by Chen & Inbar (1993).

To minimize oxidation on extraction process, a number of scientists recommended extraction of HA by NaOH in an atmosphere of $N_2$ gas. It has been shown that there was not significantly different between extraction conducted by using NaOH solution under air and under $N_2$ atmosphere. Tan *et al*. (1991) reported that $N_2$ gas can be replaced by air to meet condition of extraction. It is obvious that the use of $N_2$ can be deleted. The speed of extraction increased because extraction by air needs fewer equipment and simpler procedures than that of by $N_2$ atmosphere. The amount of extractable HAs concentration depends on origin and type of the SOWs. Similar results have been reported by Martin *et al.* (1998) and Goenadi *et al*. (2000). The HAs concentration of EFBOP and TCR composts were higher than that of CPH.

Gel chromatography is a simple and relatively effective method in achieving molecular fractionation. Tan & Giddens (1972) reported that with Sephadex G-50 filtration, HA was separated only into two fractions: a high (>30,000) and a low (<15,000) molecular weight fraction. It can be noticed that HAs with high molecular weights (MW > 30.000) have lower ratio $E_4/E_6$ values (4.32-4.45) than HAs with lower molecular weight (MW=15,000). The lower molecular weight fractions exhibit $E_4/E_6$ values of 5.47-5.49. Low molecular weight components of humic substances have proved to be biologically active, because they are absorbed slightly better by plants and show higher metabolic activity than the higher ones (Garcia *et al*., 1994). The purifcation on this experiment indicated that EFBOP, CPH, and TCR had a low molecular weight with $E_4 : E_6$ ratio 5.0 (EFBOP), 7.4 (CPH), and 7.3 (TCR) respectively (Figure 1 and 2). These results are in agree with those of Martin *et al.* (1998) on purification of HAs from natural forest soils of Sikkim ($E_4$:$E_6$ = 4.12 – 7.05), but was different with the values reported by Zhang *et al.* (1988) who found the ratio to be always less than 5.0. The differences could be attributed to differences in type of HAs.

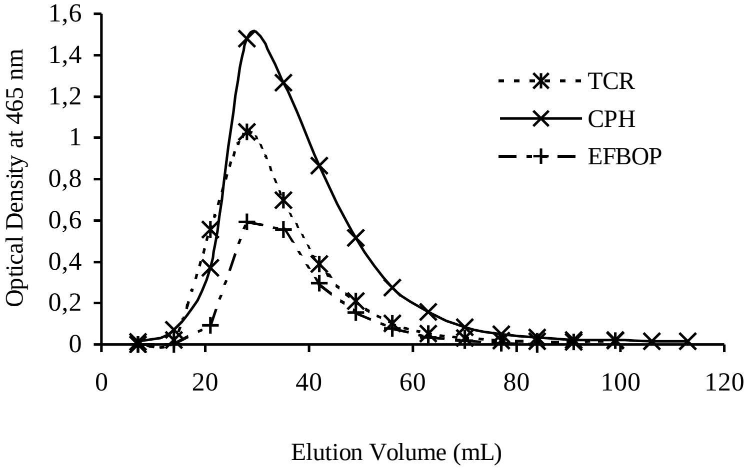

Figure 1. Purification of HA from composts of empty fruit bunches of oil palm (EFBOP), cocoa pod husks (CPH), and tea cutting residues (TCR) by using Sephadex G-50 column, OD at λ 465 nm.

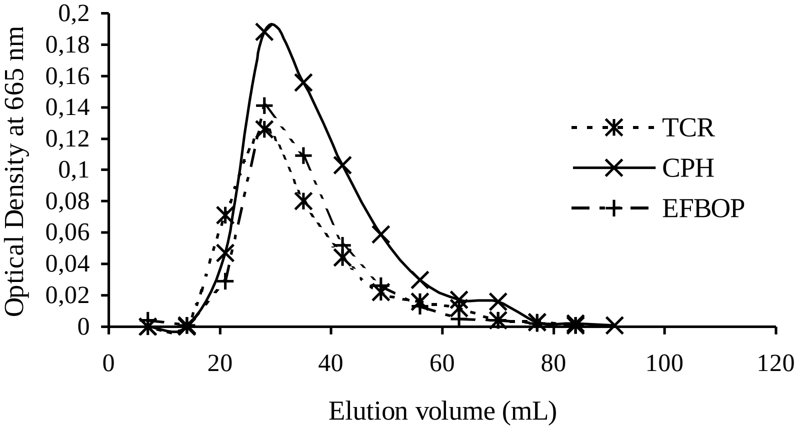

Figure 2. Purification of HA from composts of empty fruit bunches of oil palm (EFBOP), cocoa pod husks (CPH), and tea cutting residues (TCR) by using Sephadex G-50 column, OD at λ 665 nm.

*.Characteristics of HAs from plantation's SOWs*

HA elements from EFBOP, CPH and TCR composts may have a high potential on the plant growth. The main elements of HAs from EFBOP, CPH, and TCR are carbon and oxygen (Table 1). The similar results have been reported by Goenadi (1997) on HA extracted from Histosols (peat soils) and Tan (1998) from tropical and temperate region soils. However, the carbon elements of EFBOP, CPH, and TCR composts were lower than that of carbon elements in oxidized coal and Leonardite (516.0-642.0 g.kg$^{-1}$) as prenouslyt reported by Piccollo *et al.* (1992).

HA from EFBOP, CPH, and TCR composts containing large variety of amino acids in which the types and numbers are dependent on crop types. The predominant amino acids on the three SOWs investigated are aspartic and glutamic acids (Table 2). Aspartic and glutamic acids have similar structure, differing only in their number of $CH_2$ fraction and $COO^-$. The amino acid concentration of TCR compost was relatively higher than that of EFBOP and CPH. Orlov (1985) reported that most of the amino acids found in the HA was predominated by aspartic acid-serine-glycine, whereas Coelho *et al.* (1985) indicate that aspartic acid, glycine, and glutamic acids are the major components of amino acids in the soil. The presence of amino acids in the HA structure offers the possiblility of using these substances as a source of organic nitrogen to both microbes and plants.

Infrared spectroscopy has been used extensively in the past to characterize HAs and very useful to identify three different types of humic fractions. The absorption spectra of HA samples extracted from different solid organic wastes of plantation (EFBOP, CPH, and TCR) indicate the dominant of O-H, C-H, and C=O fractions. All the spectra showed a strong absorption band at 3400 - 3300 cm$^{-1}$, 2980 - 2920 cm$^{-1}$, and 1720 -1650 cm$^{-1}$, respectively (Figure 3). Tan (1998) reported that HAs exhibits a strong absorption for C-H vibrations at 2980-2920 cm$^{-1}$, and stronger absorption for both carbonyl and carboxyl vibrations of $COO^-$ form at 1720 and 1650 cm$^{-1.}$ No absorption band at 1000 cm$^{-1}$. Therefore, absorption band at 1000 cm$^{-1.}$ that occurred on SOWs of EFBOP and CPH assumed as silica gel contamination. The structure of absorption spectra of HA from that of plantation's SOWs were similar, differed only in their intensities of absorption.

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Table 1. Elemental compositions (g. $kg^{-1}$)) of HA from EFBOP, CPH, and TCR composts.

| Origin | Carbon | Hydrogen | Nitrogen | Sulphur | Oxygen |
|---|---|---|---|---|---|
| | Concentration (g.$kg^{-1}$) | | | | |
| EFBOP | 490.8 | 69.8 | 12.0 | 1.8 | 425.6 |
| CPH | 432.6 | 50.4 | 41.0 | 3.7 | 472.3 |
| TCR | 471.0 | 57.8 | 50.6 | 4.1 | 416.5 |

Table 2. Amino acid concentration (g.$kg^{-1}$) in HA from EFBOP, CPH, and TCR composts.

| Types of amino acid | EFBOP | CPH | TCR |
|---|---|---|---|
| | Concentration (g.$kg^{-1}$) | | |
| Aspartic acid | 3.4 | 19.7 | 27.1 |
| Glutamic acid | 4.4 | 24.5 | 31.8 |
| Serine | 0.7 | 4.8 | 6.6 |
| Histidine | 0.5 | 3.9 | 5.0 |
| Glycine | 1.6 | 8.3 | 12.4 |
| Threonine | 0.8 | 4.4 | 6.1 |
| Arginine | 0.9 | 6.1 | 7.3 |
| Alanine | 2.2 | 10.0 | 15.7 |
| Tyrosine | 0.9 | 5.6 | 7.6 |
| Methionine | 0.4 | 2.6 | 4.4 |
| Valine | 2.1 | 10.8 | 15.7 |
| Phenylalanine | 2.0 | 9.7 | 15.4 |
| Leucine | 1.8 | 9.5 | 13.2 |
| Isoleucine | 3.1 | 14.6 | 23.5 |
| Lysine | 0.9 | 5.3 | 6.3 |

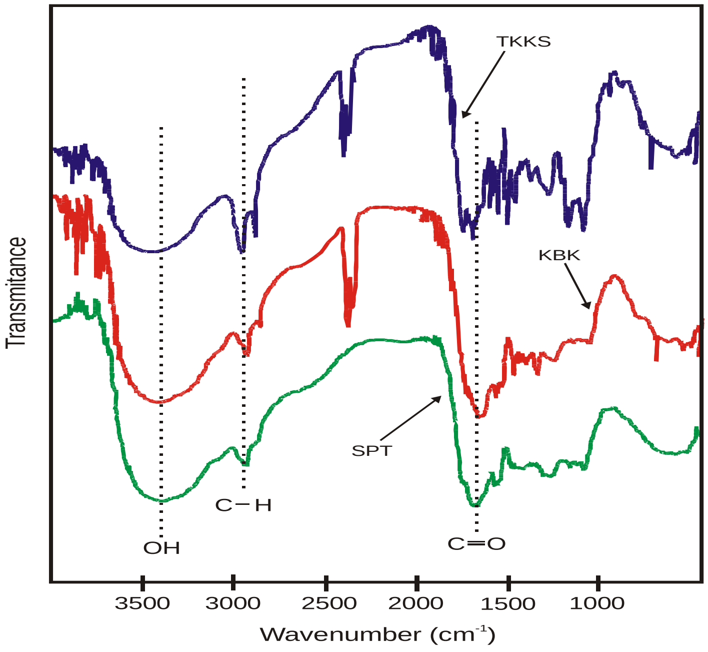

Figure 3. Fourier-Transform Infrared (FTIR) analyses in HA extracted from composts of empty fruit bunches of oil palm (EFBOP), cocoa pod husks (CPH) and tea cutting residues (TCR).

## Conclusions

The effective extraction technique of HAs acids from SOW composts of EFBOP, CPH, and TCR has been confirmed. By using conventional method using NaOH solution under air condition, the procedure can be simplified and need only a few equipments. The amount of HA from EFBOP and TCR composts were higher than that of CPH compost. The composting period and extraction method either under air or $N_2$ gas were not significantly affected the amount of the extracts. The EFBOP, CPH, and TCR composts had HAs acids with a low molecular weight and predominated by aspartic and glutamic acids. HAs from TCR compost have higher concentrations of amino acid and carboxylic compounds. The absorption spectra of the three plantation's SOW composts were similar. Based on the characteristics and high content of HA, EFBOP and TCR may be very potential for the commercial production of HA. However, the role of HAs on crops and microbes needs further study.

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