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DECREASE OF OXYGEN INTERFERENCE ON HUMIC ACID STRUCTURE ALTERATION DURING ISOLATION

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Abstract. *When dealing with a soil source, separation of humic and fulvic acids (HA and FA) from humin is usually obtained by alkaline extraction. Undesirable feature of this isolation method is that, under alkaline conditions, oxidation of HA occurs, in contact with air. This is, in part, overcome by isolation in* N_2 *atmosphere (IHSS method). However, for isolation of even less altered material, the presence of* O_2 *in the very alkaline extraction solution (AES) should not be neglected, although it usually is. In this paper we investigated the effect of* $MnSO_4$*, on* O_2 *level decrease in AES (modified IHSS method). HAs isolated by IHSS and modified IHSS methods were characterized by oxygen containing functional group determination, UV/VIS spectrophotometry and FT-IR spectrophotometry. Obtained data have shown that HA isolated by modified IHSS method, with deoxygenated AES, has less altered structure***.**

1. INTRODUCTION

Humic substances (HS) are the major organic constituents of soil, formed through plant and animal tissues breakdown by chemical and biological processes. They are widely distributed over the earth's surface and occur in almost all terrestrial and aquatic environments.

There is an increasing interest in HS research, because of their high complexing capacity for metal ions [1], involvement in the organic geochemical cycle, ability to generate various cancerogenic chlorinated products in the chlorination water treatment [2], affecting bioavailability and ecological effects of nutrients in water [3], natural elements like Fe, Al and Pb and contaminants and affecting plankton metabolism [4].

Despite extensive research concerning HS formation and its environmental impact, the chemical structures of HS are still not known with a desirable level of certainty. In general, HS are described as a mixture of naturally occuring, polydisperse, heterogeneous

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polyelectrolytes [5], that can be divided into three fractions, on the basis of their aqueous solubility, into:

- humic acid (HA) fraction (soluble at $pH \geq 6.0$),
- fulvic acid (FA) fraction (soluble at all pHs) and
- humin (insoluble at all pHs).

Before HS can be effectively studied, they must be separated from the bulk of other organic and inorganic constituents present in soil and water, and concentrated. While development and improvement of HS isolation method go back more than 200 years, much remains to be learned about it. The ideal extraction method is the one which meets the following objectives: a) the method should lead to the isolation of unaltered material; b) the extracted HS should be free of inorganic contaminants, such as clay and polyvalent cations; c) extraction should be complete, thereby insuring representation of fractions from the entire molecular-weight range and d) the method should be universally applicable to all soils.

There is a great number of methods for extraction of soil HA, using different extraction reagents, which are summarized in table 1.

Table 1. Reagents usually used for extraction of organic constituents from soil [5]

Type of material	Extractant	Organic matter extracted
Humic substances	NaOH	to 80%
	Mild extractants:	
	$Na_4P_2O_7$ and other	to 30%
	Organic chelates: acetyloacetone, cupferron, hydroxyquinoline	to 30%
	Formic acid (HCOOH)	to 55%

From these data, it is evident that, extraction of HS from soil with NaOH solution leads to the recovery of approximately two-thirds of the soil organic matter. It is known that the amount of organic matter extracted from soil with caustic alkali increases with time of extraction, but the longer the extraction period and the more alkaline solution, the greater will be the chemical changes.

In general, disadvantages of alkali extraction are as follows: a) alkali solutions dissolve silica from the mineral matter and this silica contaminates the organic fractions separated from the extract; b) alkali solutions dissolve protoplasmic and structural components from fresh organic tissues and these become mixed with the humified organic matter; c) under alkaline conditions, autoxidation of some organic constituents occurs in contact with air both during extraction and when the extracts are allowed to stand; d) in alkaline solution condensation between amino acids and aldehydes or quinones can occur.

So, it is obvious that contact with air, when HS material is under alkaline condition, should be avoided. This is, in part, overcome by isolation in N_2 atmosphere, like in IHSS isolation procedure (International Humic Substances Society) [6]. However, for isolation of even less altered material, the presence of O_2 in the alkaline extraction solution (AES) should not be neglected, although it usually is. Therefore, we modified standard IHSS procedure by using $MnSO_4$ for deoxigenation of AES. Dissolved oxygen present in the AES, rapidly oxidizes dispersed divalent manganous hydroxide precipitate to hydroxide of higher valency state, as shown in the equation (1):

$$Mn^{2+} + OH^{-} \rightarrow Mn(OH)_2 + O_2 \rightarrow MnO(OH)_2 \qquad (1)$$

This deoxygenised AES is, after filtration in N_2 atmosphere, used for HA extraction.

In order to investigate the effect of oxygen decrease on isolation of less altered HA structure, HA were isolated in three ways: by deoxygenated AES under N_2 atmosphere (IHSS-N_2/Mn method), by non-deoxygenated AES under N_2 atmosphere (IHSS-N_2 method) and finally by non-deoxygenated AES in air (IHSS-O_2 method), in which oxygen level was increased, respectively. Isolated HAs were characterised by oxygen containing functional groups determination, ultraviolet/visible spectrophotometry (UV/VIS) and Fourier Transform Infrared spectrophotometry (FT-IR).

2. EXPERIMENTAL

All solutions were prepared with water that was doubly deionised and distilled from $KMnO_4$ solution (DD-water). Analytical grade reagents were used to prepare all solutions. Glass apparatus and vessels were cleaned by soaking in 10% HNO_3 followed by rinses with distilled water.

Soil sample preparation. The soil sample was obtained from a well-humified organic horizon of old beech-forest soil (10 cm depth), in autumn 1998, using standard grinding equipment. The soil was air-dried and sieved to pass a 2.0 mm sieve.

Extraction and purification of HA. The procedure used for separation and purification of HA was based on the method described by IHSS [6]. An overview of the modified IHSS isolation and purification procedure (IHSS-N_2/Mn method) is given in Fig. 1.

In initial treatment, phosporic acid was used rather than HCl, which is recommended by IHSS, because H_3PO_4 would prevent the oxidation of organic substances by the Fe(III) ion [7]. Also, AES was deoxigenized by dissolving 2.0 g $MnSO_4$ in 1000ml NaOH solution. After 12h, the solution was filtered, under N_2 atmosphere. All experiments in alkaline media were carried out in closed chamber under low speed of N_2, in order to decrease O_2 level as much as possible. Bound metal ions were removed form HA by passage over ion-exchange resin Dowex 50W-X8 in the following procedure: HA was dissolved in 0.1M KOH (prepared with DD-water in which N_2 was purged in order to remove dissolved O_2), in a separating funnel of appropriate volume so that no air was left over the solution surface. The funnel was closed, via stopcock connected with N_2, and put on a 300x10 mm column of Dowex 50W - X8 (H^+ form) at a flow rate of 0.5ml/min. HA was passed over Dowex and collected in a flask (in which a minute volume of H_2SO_4 was added) connected with N_2. HA rapidly coagulates in acid solution. The protonated HA was centrifuged, dried at 30°C and left over $CaCl_2$.

Beside HA isolation by above descibed IHSS-N_2/Mn method, HA were isolated by standard IHSS-N_2 method [6] and finally by IHSS-O_2 method (the same as IHSS-N_2 method, but without N_2 atmosphere). Spectral data for all three HAs were than compared.

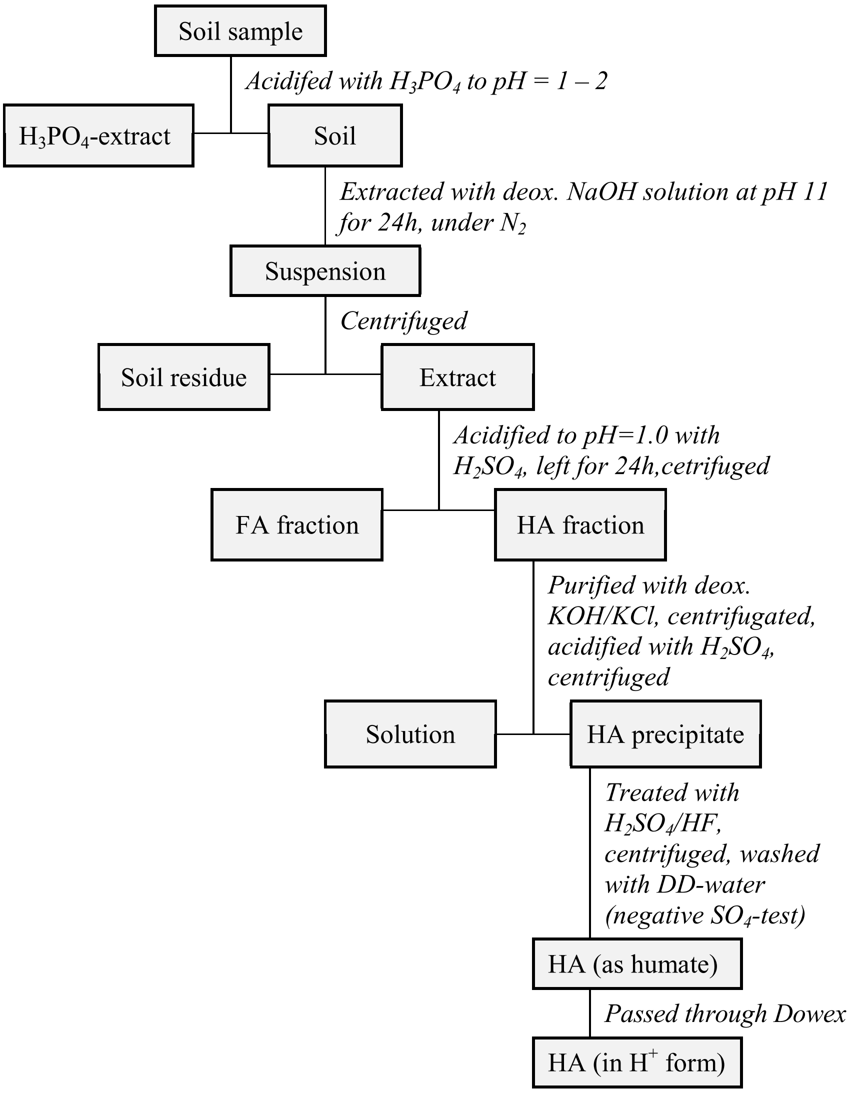

Fig. 1. Experimental scheme for soil HA isolation method (IHSS-N_2/Mn method)

Elemental analysis. The carbon, hydrogen, nitrogen and sulphur content of the HA sample were determined directly, while oxygen was obtained by difference.

UV/VIS spectroscopy. UV/VIS spectra of all three HAs (4 mg) dissolved in 100 ml 0.05M $NaHCO_3$ solution were recorded on a Secomam Anthelie 306 spectrophotometer, using wavelengths ranging from 200 to 1000 nm.

FT-IR characterisation. FT-IR spectra of all three HAs were recorded on KBr pellets (0.5 mg HAs and 150mg KBr) using a Bomem Hartman & Braun MB-Series FT-IR spectrophotometer.

Oxygen-containing functional groups determination. Total acidity, carboxyl and phenolic groups content were determined by methods described by Schnitzer [1].

3. RESULTS AND DISCCUSION

Elemental analysis and atomic ratios of HA isolated by IHSS-N_2/Mn method. The elemental composition of the HA isolated by IHSS-N_2/Mn method is given in table 2. Values are in the range typical for HA of soil origin, according to the statistical evaluation of elemental composition of 215 soil HA samples [8].

Table 2. Elemental composition of HA isolated by IHSS-N2/Mn method and literature values [8]

		Isolated HA	Literature values for HA	
			mean	range
Elemental content	C	44.86	55.40	37.18–64.10
	H	4.82	4.80	1.64–8.00
	N	2.66	3.60	0.50–7.00
	S	0	0.80	0.1–4.88
	O*	47.66	36.00	27.1–51.98
C/N		19.67	–	–
H/C		1.29	1.04	0.08–1.77
O/C		0.80	0.50	0.33–0.97
Ash, wt %		2.69	–	–

[*] Estimated as the difference from 100%

UV/VIS spectra of HAs. HA gives relatively featureless spectra without maxima or minima, in the UV- and VIS-region (Fig. 2).

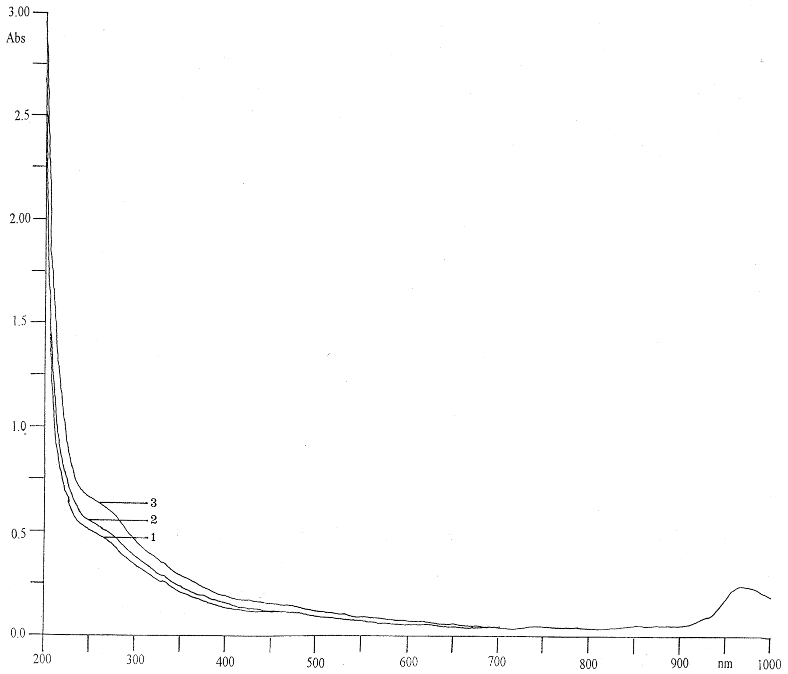

Fig. 2. UV/VIS spectra of terrestrial HAs: (1) HA isolated by IHSS-N_2/Mn method, (2) HA isolated by IHSS-N_2 method, and (3) HA isolated by IHSS-O_2 method

The absorption in the UV region is mainly caused by the excitation of electron lone pair, usually oxygen (n→π*) and by conjugated C = C double bonds (π→π*). The absorption in the VIS region is caused by lone pair electrons and to charge-transfer systems. The absorbance decreases as the wavelength increases, as typical for HS [1,5]. A slight maximum could be indicated at approximately 275 nm, which is probably due to quinone structure [1] and at 960 nm probably due to overtone lines of the O-H streching vibratrions of phenols, alcohols or acids or various N-H streching vibrations and their overtones of primary and/or secondary amines. It is also indicated that spectral absorption, at 275 nm, is different for all three HAs, and rise in the following order: HA isolated by IHSS-N_2/Mn method (lowest), HA isolated by IHSS-N_2 method (intermediate) and HA isolated by IHSS-O_2 method (highest). Therefore, oxidation of easily oxidisable phenolic groups to quinone groups probably does not occur when extraction was done with deoxygenated AES [9]. Also, the ratio of absorbencies at 465 nm and at 665 nm, referred to as E_4/E_6 ratio, which is often used for the HS characterisation [1], varies for isolated HAs. Table 3. shows that increase of oxygen level during extraction from IHSS-N_2/Mn method to IHSS-O_2 method, increase E_4/E_6 ratio, as well. In order to approve this correlation, current of CO_2-free air was bubbled through alkali solution of HA at pH 11 for 4 hours. UV/VIS spectra of this "experimental" HA was recorded and compared with UV/VIS spectra of "control" HA (alkali solution at pH 11, without passage of air) (Fig. 3). Absorbencies and E_4/E_6 ratios for control and experimental HA show the same trend as described above (see table 3.).

Table 3. Absorbances and E_4/E_6 ratios for HAs isolated by IHSS-N_2/Mn, IHSS-N_2 and IHSS-O_2 methods

λ (nm)	HA isolated by method			Control HA**	Experimental HA*
	IHSS-N_2/Mn	IHSS-N_2	IHSS-O_2		
254	0.4957	0.5455	0.6595	1.6212	2.0198
465	0.1073	0.1137	0.1445	0.3153	0.3693
665	0.0408	0.0419	0.0486	0.0896	0.1008
E_4/E_6	2.63	2.71	2.97	3.51	3.66

* current of CO_2-free air bubbled through alkali solution of HA at pH 11

** alkali solution of HA at pH 11 without exposure to air

Oxygen-containing functional groups of HAs. Table 4. shows the distribution of oxygen containing functional groups in HAs isolated by the described methods. Obtained values are in the range typical for HAs of soil origin [1]. It is notable that there is a decrease in total acidity and content of phenolic hydroxyls with the increase in oxygen exposure during extraction. Differences between obtained values are especially high for easily oxidisable phenolic hydroxyls.

Table 4. Distribution of oxygen-containing functional groups (meqg^{-1}) in HAs

	HA isolated by method		
	IHSS-O_2	IHSS-N_2	IHSS-N_2/Mn
Total acidity (meq g^{-1})	4.17	5.81	7.41
COOH (meq g^{-1})	3.20	3.40	3.30
Phenolic OH (meq g^{-1})	0.97	2.41	4.11

FT-IR spectra. In contrast to the featureless UV/VIS spectra, the solid state FT-IR spectra of HA shows many bands which are characteristic for specific molecular bonds (Fig. 4).

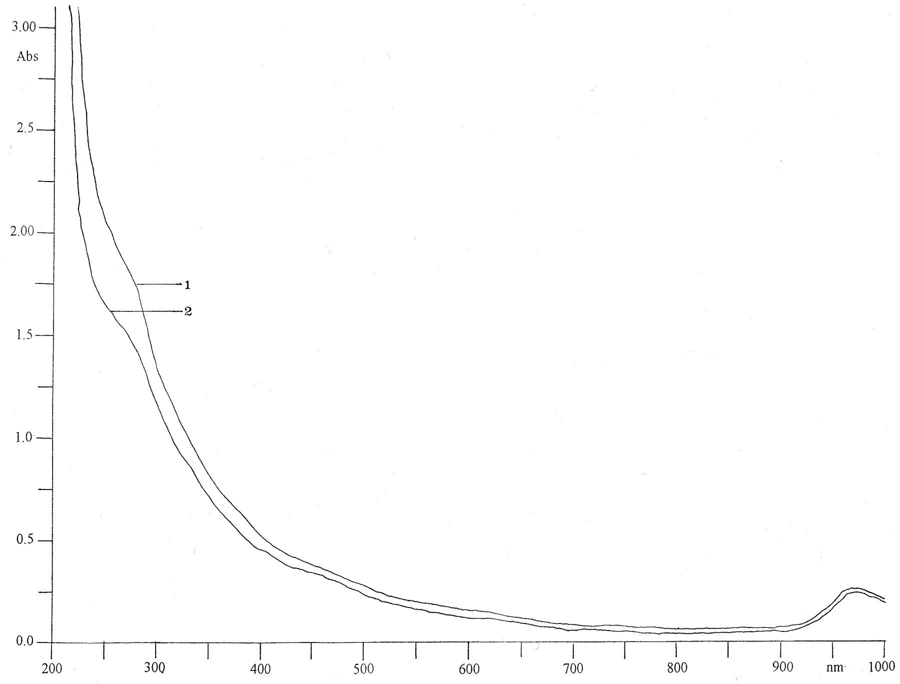

Fig. 3. UV/VIS spectra of terrestrial HAs isolated by IHSS-N_2/Mn method: (1) experimental HA, (2) control HA

Interpretation of the humic acid absorption bands was done as described in the literature [10]. FT-IR spectra was dominated by absorbencies due to aromatic, aliphatic, carboxylic and hydroxylic groups mainly. The strong band at 3400-3300cm^{-1} reflects the OH-groups of alcohol, phenols and carboxylic acids. Due to the usually low N content in HA, amines and amides do not appear in the spectra. The absorption bands at 2927 and 2853 cm^{-1} are caused by C-H stretching of methyl and methylene groups. Two of the main bands appear at 1721 cm^{-1} and 1639 cm^{-1} due to the stretching frequencies of C=O bonds of carbonyl and carboxyl, and due to C=C vibration of aromatic and aliphatic molecular units conjugated with C=O. The fingerprint region (below 1500cm^{-1}) is difficult to assign because of a wide variety of combination bands. The IR spectra of three isolated HAs shows some negligible differences. The contribution of aliphatic structures is fairly high in the case of HA isolated by deoxygenated AES (there is one more band at 2853 cm^{-1}).

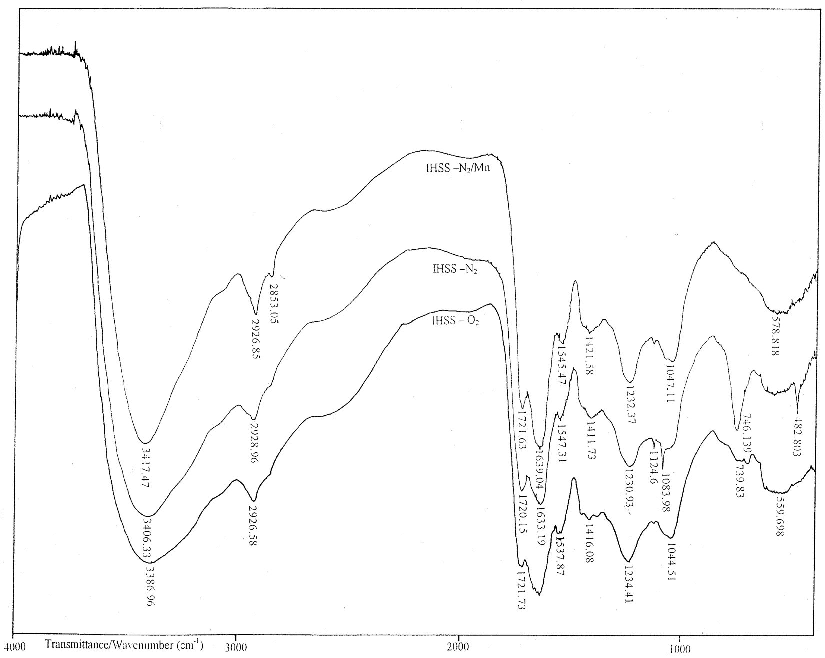

Fig. 4. FT-IR spectra of terrestrial HAs
isolated by IHSS-N$_2$/Mn, IHSS-N$_2$ and IHSS – O$_2$ methods

3. CONCLUSION

Terrestrial HAs were isolated and purified by three methods: by deoxygenated AES under N$_2$ atmosphere (modified method, IHSS-N$_2$/Mn method), by non-deoxygenated AES under N$_2$ atmosphere (IHSS-N$_2$ method) and finally by non-deoxygenated AES in air (IHSS-O$_2$ method). In those methods exposure of HA to oxygen during extraction is increased, respectively. Estimation of oxygen interference on HA structure was done by UV/VIS and FT-IR characterisation and by oxygen containing functional groups determination. Values for E$_4$/E$_6$ ratios, total acidities and phenolic hydroxyls contents are the highest for HA isolated by modified method which indicates that this HA posses less altered ease oxidisable phenolic hydroxyl groups. Concerning differences in the obtained data of all three HAs, it is reasonable to assume that significant alteration occurs in the cases when MnSO$_4$ is not used in the isolation procedure. So, it is recommended to use this modified, IHSS – N$_2$/Mn method in isolation of HA with more preserved structure.

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SMANJENJE INTERFERIRAJUĆEG UTICAJA KISEONIKA NA ALTERACIJU STRUKTURE HUMINSKIH KISELINA TOKOM IZOLOVANJA

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Terestrične huminske kiseline (HA) se obično izoluju metodom alkalne ekstrakcije. Pod ovim alkalnim uslovima, lako dolazi do oksidacije HA vazdušnim kiseonikom. Da bi se ovaj nedostatak alkalne ekstrakcije smanjio, izolovanje se obično vrši pod azotom., pri čemu se zanemaruje prisustvo kiseonika i u samom ekstrakcionom rastvoru. U ovom radu je ispitan efekat MnSO4, na smanjenje količine prisutnog kiseonika u ekstrakcionom sredstvu. Standardna metoda izolovanja HA je modifikovana tako što je korišćen deoksigenovani alkalni ekstrakcioni rastvor. S ciljem da se ispita efikasnost MnSO4 u sprečavanju moguće alteracije strukture HA, izolovana je HA na vazduhu, zatim pod azotom i na kraju pod azotom ali u prisustvu MnSO4. Nakon izolovanja HA, izvršena je njihova karakterizacija elementarnom analizom, analiziran je sadržaj kiseoničnih funkcionalnih grupa i snimljeni su njihovi UV/VIS i FT-IR spektri. Dobijeni rezultati pokazuju da se sa povećanjem nivoa kiseonika pri izolovanju, struktura kiseline menja. Najveći efekat prisutan kiseonik pokazuje na sadržaj lako oksidujućih fenolnih hidroksilnih grupa, kojih ima najviše kod HA izolovane po modifikovanoj metodi, što pokazuje da do oksidacije HA ili uopšte ne dolazi ili se ona odvija u najmanjoj mogućoj meri.