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Identification of Soybean Husk and Cow Manure Metabolites after Vermicomposting

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***Abstract*— Soybean husk and cow manure are organic wastes that can be used as raw materials for making organic fertilizers through vermicomposting technique. To explore the potential, it was necessary to identify the vermicompost metabolites produced. Gas Chromatography-Mass Spectroscopy (GC-MS), a powerful tool, was used to identify metabolites that characterize vermicompost. The purpose of this study was to identify vermicompost metabolites derived from different proportions of soybean husk and cow manure. Information obtained can guide alternative soybean husk and cow manure use, especially as raw materials for organic vermicompost fertilizer. The materials used in this study consisted of soybean husk, cow manure and lumbricus rubellus earthworms. This vermicomposting study was conducted using a Randomized Complete Block Design (RCBD) with five treatments and five replicates. The treatment details are: V_1 = soybean husk (100%); V_2 = cow manure (100%), V_3 = soybean husk : cow manure (50%: 50%); V_4 = soybean husk : cow manure (75%: 25%) and V_5 = soybean husk : cow manure (25%: 75%). Metabolites were analyzed using GC-MS and the Least Significant Difference (LSD) test. Based on GC-MS analyses of all treatments (V_1-V_5), metabolites were identified consist of sugar compounds, amino acid compounds, organic acids, vitamins, and hormones. Consistent relationships between chromatograms of treatment V_1-V_5 were directly proportional to the LSD test results; treatment V_5 consistently yielded the highest total area under the curve.**

***Keywords*—Vermicompost; soybean husk; cow manure; GC-MS.**

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I. INTRODUCTION

Soybean-husk is an organic waste by-product obtained by dehusking soybeans in the soy-based foods processing industries. Examples of soy-based foods include tempeh, tofu, soy sauce, soy oil, and soy milk [1]–[3]. Soybean husks are a rich dietary fiber source comprising 86% polysaccharides, proteins, lipids, vitamins, minerals, and polyphenols such as anthocyanidins and proanthocyanidins, and isoflavones. [4]. Soybean husk has the potential to be used as organic fertilizer. This potential was confirmed by the results of a soybean husk and papaya waste vermicomposting trial, which showed a significant increase in the P, K, Ca, and Mg as well as a decrease in C/N ratio [2]. These chemical characteristics indicate that soybean husk could potentially be used as organic fertilizer. In addition to chemical characteristics, it was necessary to identify other characteristics, namely the identification of metabolites produced. Metabolites could help identify cell mechanisms and metabolic profiles, which in turn could help decipher the organism's physiological and biological functions and metabolic pathways and could unlock the characteristics of soybean husk. To date, few studies have reviewed soybean husk as a raw material for making organic fertilizer or looked at the metabolites derived from this process.

This study was conducted to identify metabolites derived from soybean husk composted in combination with cow manure at different proportions using a vermicomposting technique. Vermicomposting is a method of bioconverting organic waste into organic fertilizer that can be used as a substrate for plant growth using earthworms and microorganisms [5]–[11] known as vermicompost, which was a solid product of vermicomposting, characterized by brown color and odorless. The advantage of vermicomposting, compared to other composting techniques, is the decomposer

(an earthworm). Earthworms release enzymes such as protease, lipase, amylase, cellulose, and chitin that catalyze chemical reactions in cellulose and protein [12], [13] earthworms process the cellulose in the waste that compost bacteria cannot break down; the produced vermicompost is superior to ordinary compost. An organic fertilizer having a nutrient-rich profile and plant growth hormones increases plant growth, revitalizes soil quality, maintains moisture, and provides nutrients [5], [14]–[16]. The earthworms defined the quality of vermicompost as decomposers and the raw materials (waste) being vermicomposted. Vermicompost quality varies with the combinations of waste types composted (livestock manure with agricultural, industrial and/or household waste food processing waste, and market waste such as fruits and vegetables) [7], [17]–[19]. However, no studies on soybean husk combined with livestock manure have been conducted.

Cow manure is used in vermicomposting because cow manure contains a high diversity of microorganisms and also has the highest fiber content (cellulose) compared to other livestock manures (C/N ratio > 30). This condition supplies the earthworms with additional energy; cow manure is a good substrate to mix with other wastes in vermicomposting [6], [20]–[22]. Varying the proportions of raw materials (soybean husk and cow manure) results in different vermicompost characteristics, including metabolites produced. The identification of metabolites from vermicompost in this study was conducted using Gas Chromatography-Mass Spectroscopy (GC-MS), a powerful tool for characterizing vermicompost [23],[24]. GC-MS can also be used to separate and detect polar compounds (organic acids, amino acids, sugars, and sugar alcohols) and non-polar compounds (fatty acids and sterols) and exhibit retention indexes and mass spectrums [25]. This study aimed to identify metabolites in vermicompost made from soybean husk combined with cow manure. The information obtained could be used to make recommendations to soybean husk managers on the applicable uses of the waste in vermicomposting. Over the long term, inorganic (synthetic) fertilizers could be replaced by organic fertilizers (vermicompost) for a reasonable cost, thus assuring food and environmental security and increasing the use of environmentally friendly, sustainable agricultural production processes.

II. Materials and Methods

A. Research Setting

The study was conducted at the Green House Garden of the University of Muhammadiyah Malang, Indonesia. The vermicomposting material consisted of soybean husk obtained from tempeh producers in Sanan, Malang, East Java, cow manure, and *Lumbricus rubellus* earthworms. The vermicomposting study was conducted in a Randomized Complete Block Design (RCBD) with five treatments and five replications. The treatment details were as follows: V_1 = soybean husk (100%); V_2 = cow manure (100%); V_3 = soybean husk: cow manure (50%:50%); V_4 = soybean husk: cow manure (75%:25%), and V_5 = soybean husk: cow manure (25%:75%).

The experiment's implementation started with washing the soybean husk until the water was clear, reducing the acidity of the waste. Soybean husk was then dried by aerating while the cow manure was obtained from dairy farmers. The dried soybean husk was mixed with cow manure according to the following predetermined (soybean husk: cow manure) proportions: 50%:50%, 75%:25%, and 25%:75%. Proportions were measured by weight. The four treatments, a 100% soybean husk treatment and a 100% cow manure treatment, were anaerobically fermented for two weeks. Treatments were then transferred to twenty-five boxes, sized 40 cm x 40 cm x 20 cm, for vermicomposting. 10 kg of the anaerobically fermented treatment material and 1000 g of L. rubellus worms were added to each box. The vermicomposting process spanned seven weeks and produced a smooth, blackish vermicompost material. The vermicompost treatments were analyzed using GC-MS. A Least Significant Difference (LSD) test was performed to analyze the differences between treatments.

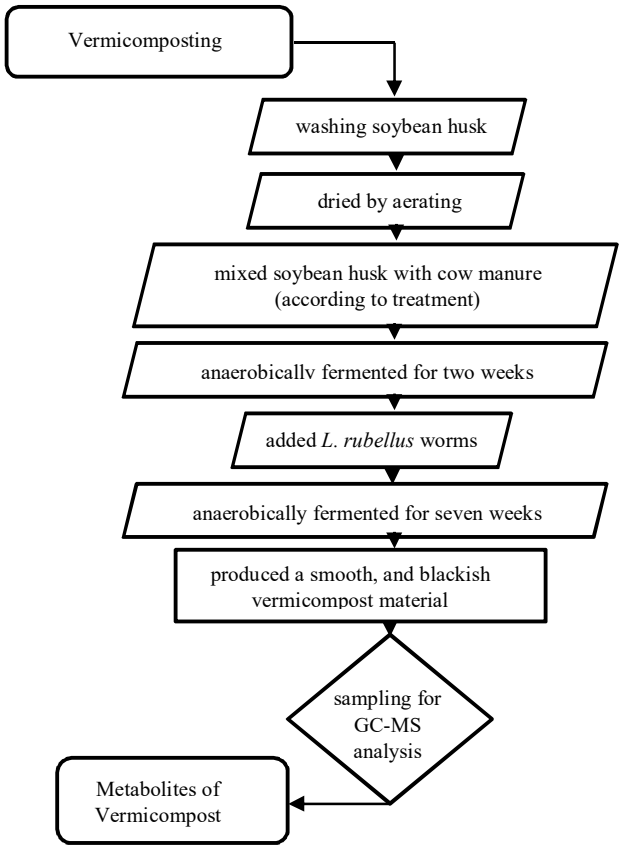

Fig. 1 Vermicomposting from soybean hust and cow manure

B. Sampling for GC-MS analysis

Random sampling was completed on all replication boxes. The vermicompost samples were dried to a constant weight, smoothed using a blender, and sieved with a 40-mesh sieve. An analytical scale was used to weigh 5 grams of each sample. The samples were then transferred into 250 ml beakers and 50 ml of absolute methanol [26],[27] and stirred with magnetic stirrers for 30 minutes. The resulting solutions were filtered, and the residue was relocated into Erlenmeyer 250 ml flasks; 50 ml of absolute methanol was then added to each sample and stirred with magnetic stirrers for 30 minutes. The stirred solution was re-filtered, producing a clear filtrate. The filtrate was diluted to 250 ml in a flask. Then filtrate solution was

concentrated using a rotary evaporator at 40^0C with a 150 mm Hg suction pressure, yielding a concentrated solution. A 0.1 g sample of the concentrated extract was taken and diluted with absolute methanol to 100%. Samples were homogenized with a vortex and filtered with a cellulose acetate 0.45 µm membrane. Finally, sample solution degassing was performed before its injection into the GC-MS.

III. Result and Discussion

A. Result

The GC-MS vermicompost analyses identified sugar metabolites, amino acids, organic acids, vitamins, and hormones. Treatment test results showed that, for all metabolites, the highest chromatogram increase occurred in treatment V_5 (25% soybean husk:75% cow manure), while the lowest chromatogram increase occurred in treatment V_1 (100 % soybean husk). These results were directly proportional to results of the LSD test, where treatment V_5 (25% soybean husk:75% cow manure) also yielded the highest total area under curve and treatment V_1 (100% soybean husk) yielded the lowest total area under the curve for all metabolites. The treatment metabolite results for the best-performing treatment are presented in Table I-V.

In sugar metabolite, treatment V_5 (25% soybean husk:75% cow manure) had the highest total area of the curve, 27048.8. Treatment V_1 (100% soybean husk) had the lowest total area of the curve, 23145.1. Treatment V_2 (100% cow manure) had a total area under the curve of 24145.1, V_3 (50% soybean husk:50% cow manure) was 25257.4, and V_4 (75% soybean husk:25% cow manure) was 25699.1. For amino acid metabolites, treatment V_1 (100% soybean husk) had the lowest total area of the curve (14148.1). Chromatogram areas increased incrementally with each treatment, the total area under the curve for treatment V_2 (100% cow manure) was 16003.8; followed by treatment V_3 (50% soybean husk:50% cow manure) at 18100.4; treatment V_4 (75% soybean husk:25% cow manure) at 20327.9; and treatment V_5 treatment (25% soybean husk:75% cow manure) at 22094.3

TABLE I
SUGAR COMPOUND

Identification of Compounds	Retention Time (Minute)	Area of Curves (%)				
		V_1	V_2	V_3	V_4	V_5
Raffinose	41.8	2303.4	2417.6	2532.9	2070.4	2783.2
Stachyose	68.7	4143.6	4057.8	4573.2	4690.5	3203.2
Glucosamine	5.3	693.7	1208.0	1723.3	2640.8	3253.6
Glucose	5.5	2523.5	2437.8	2553.1	2670.5	2983.3
Maltose	15.3	4631.3	4845.6	5060.9	5278.3	5391.2
Trehalose	15.3	5858.5	6072.8	5588.1	5705.5	5918.3
Arabinose	4.7	2991.1	3105.4	3225.7	2643.1	3515.9
Total		23145.1	24145	25257.2	25699.1	27048.7

TABLE II
AMINO ACID COMPOUND

Identification of Compounds	Retention Time (Minute)	Area of Curves (%)				
		V_1	V_2	V_3	V_4	V_5
Alanine	1.9	569.5	643.8	729.1	806.5	879.3
Arginine	5.2	980.2	1124.5	1259.8	1477.2	1530.0
Asparagine	4.0	426.8	481.1	546.5	663.9	666.7
Aspartic acid	4.2	842.2	966.4	1081.8	1299.1	1311.9
Cystein	2.6	286.8	321.1	366.4	383.8	446.6
Glutamic acid	4.5	1536.6	1750.9	1986.2	2203.6	2416.4
Glutamine	4,3	849.9	964.1	1089.5	1206.8	1319.7
Glycine	1.6	416.7	481.0	546. 3	603.7	666.5
Histidine	4.9	779.4	803.7	908.9	1006.4	1119.2
Isoleucine	3.9	1129.9	1244.2	1449.5	1666.9	1779.7
Leucine	3.9	841.6	965.9	1081.2	1098.6	1311.4
Lysine	4.3	981.3	1125.6	1260.9	1418.3	1531.2
Methionine	4.6	562.5	646.8	722.1	839.5	882.3
Phenylalanine	4.9	709.4	803.6	908.9	1026.4	1139.2
Proline	2.1	286.8	301.1	366.4	403.8	446.6
Serine	1.9	276.4	320.7	366.0	383.4	446.2
Threonine	2.3	840.6	964.9	1080.3	1217.6	1330.5
Tryptophan	7.4	428.9	483.2	548.5	585.9	668.7
Tyrosine	5.9	563.2	647.4	712.8	830.2	882.9
Valine	2.1	839.1	963.4	1088.8	1206.1	1318.9
Total		14148.1	16003.8	18100.4	20327.9	22094.3

The organic acid and ester metabolites' total area of the curve for treatment V_1 (100% soybean husk) was 5161.8; treatment V_2 (100% cow manure) was 5986.3; treatment V_3 (50% soybean husk:50% cow manure) was 7035.3; treatment V_4 (75% soybean husk:25% cow manure) was 7882.8, and treatment V_5 (25% soybean husk waste 75% cow manure) was 8933.9. These results were directly proportional to the respective LSD test in that treatment V_5 showed the highest

total area of the curve, and treatment V_1 showed the lowest total area of the curve. The vitamin metabolite total area under the curve for treatment V_1 (100% soybean husk) was 2061.5; for treatment V_2 (100% cow manure) was 3030.1, for treatment V_3 (50% soybean husk:50% cow manure) was 4018.0 for treatment V_4 (75% soybean husk:25% cow manure) was 5042.5, and treatment V_5 (25% soybean husk:75% cow manure) yielded the highest total area under the curve, (6067.9). The hormone metabolites' chromatogram results were similar to other results, showing progressive increases from treatment V_1 to V_5. The total area under the curve in treatment V_1 (100% soybean husk) was 3057. 9, treatment V_2 (100% cow manure) was 3100.8, treatment V_3 (50% soybean husk:50% cow manure) was 4100.7, treatment V_4 (75% soybean husk:25% cow manure) was 5232.9, and the highest total area of the curve (6171.4) was observed in treatment V_5 (25% soybean husk:75% cow manure). Figure 2 showed that sugars were the most-dominated metabolite formed, followed by amino acids, organic acids, vitamins, and hormones. For all treatments, the most prevalent metabolite(s) formed in the sugar group were trehalose compounds, for amino acids was glutamic acid, for vitamins was myoinositol, for hormones were indole-3-butyric acid, and for organic acids and esters were α-linolenic acid.

TABLE III
ORGANIC ACID AND ESTER COMPOUND

Identification of Compounds	Retention Time (Minute)	Area of Curves (%)				
		V_1	V_2	V_3	V_4	V_5
Acetic acid	1.2	101.6	115.9	141.2	158.6	171.4
α Ketoglutaric acid	4.3	153.8	178.1	213.4	230.9	263.7
α Linolenic acid	14.6	253.5	307.8	353.1	390.5	453.3
Arachidonic acid	15.2	207.6	241.9	287.2	324.6	367.4
Caffeic acid	5.6	51.2	65.5	70.9	88.2	101.1
Chlorogenic acid	16.0	108.9	123.2	138.5	155.9	178.7
Citric acid	6.2	105.7	120.0	135.3	152.7	185.5
Ferulic acid	6.4	103.7	118.0	133.3	150.8	173.6
Formic acid	1.0	51.7	66.0	81.3	98.7	111.5
Fumaric acid	2.1	148.0	172.3	217.6	234.9	277.8
Gentisic acid	4.8	105.8	120.1	145.4	162.8	175.6
Glucuronic acid	6.3	146.4	180.7	216.0	233.4	266.2
Isocarpic acid	2.1	104.4	118.7	143.9	161.4	174.2
Isovaleric acid	1.9	49.2	63.4	78.8	96.2	108.9
Lignoceric acid	16.1	108.9	123.2	138.5	155.9	178.7
Linoleic acid	14.6	157.7	172.0	217.4	234.7	267.6
Malic acid	4.2	101.3	115.6	140.9	158.3	171.2
Myristic acid	12.9	110.0	124.3	139.6	157.0	179.8
Decanoic acid	5.0	107.4	121.7	136.9	154.4	177.2
Nonanoic acid	4.9	113.7	128.1	143.4	160.8	173.6
Hydrocinnamic acid	4.7	106.7	121.0	136.3	153.7	176.5
Oleic acid	14.6	103.4	117.7	132.9	150.4	173.2
Palmitic acid	13.8	109.8	124.1	139.4	156.8	179.6
Succinic acid	2.1	100.5	114.8	130.1	157.5	170.3
2 Methyl propionic acid	1.6	101.2	115.5	130.8	158.2	171.0
Butanoic acid	1.6	107.4	121.7	137.0	164.4	177.2
Methyl butanoate	1.9	109.0	123.3	138.6	156.0	178.8
3 Methyl butanoic acid	1.9	153.8	178.1	193.4	240.8	263.6
2 Methyl butanoic acid	1.9	108.8	123.1	138.4	155.8	178.6
Ethyl isobutanoate	2.1	109.4	123.7	138.9	156.4	179.2
Ethyl butanoate	2.1	109.7	124.0	139.3	156.7	179.5
Butyl acetate	2.1	153.7	178.1	213.4	230.7	273.6
Hexanoic acid	2.1	109.9	124.2	139.6	156.9	179.8
Propyl butanoate	3.9	150.2	174.5	229.9	247.2	270.1
Ethyl 2 Methyl butanoate	3.0	143.7	187.9	213.3	230.8	263.5
Methyl hexanoate	3.9	97.5	111.8	147.1	164.4	187.3
Heptanoic acid	3.9	153.3	177.7	202.9	220.4	263.2
Butyl isobutanoate	4.2	101.3	115.6	130.9	148.3	171.2
Butyl butanoate	4.2	140.9	175.2	210.5	227.9	260.7
Ethyl hexanoate	4.2	148.3	172.6	217.9	235.3	268.2
Hexyl acetate	4.2	101.5	115.7	141.1	158.4	171.3
Propyl hexanoate	4.9	149.1	173.4	218.7	236.1	268.9
Ethyl heptanoate	4.9	101.4	115.7	141.0	158.4	171.2
Total		5161.7	5986.3	7035.3	7882.8	8933.9

TABLE IV
VITAMIN COMPOUND

Identification of Compounds	Retention Time (Minute)	Area of Curves (%)				
		V_1	V_2	V_3	V_4	V_5
Thiamine	14.0	303.4	457.7	603.0	750.4	843.2
Riboflavine	19.9	314.7	459.0	594.4	751.7	904.6
Pantothenic acid	12.8	349.3	513.6	688.9	856.3	1029.1
Pyridoxine	5.0	271.2	365.4	440.8	508.2	620.9
Biotin	13.3	146.8	211.1	286.4	353.8	426.6
Niacin	3.8	60.5	84.8	120.2	157.5	180.4
Myo inositol	5.6	368.7	573.0	788.3	1055.7	1328.5
Nicotinamine	15.0	203.4	307.7	423.0	503.4	616.2
Total		2061.5	3030.1	4018.1	5042.5	6067.8

TABLE V
HORMONE COMPOUND

Identification of Compounds	Retention Time (Minute)	Area of Curves (%)				
		V_1	V_2	V_3	V_4	V_5
Indole 3 butyric acid	7.1	1223.6	1237.9	1653.2	2070.6	2483.4
Indole 3 acetic acid	5.2	1213.7	1227.9	1643.3	2160.8	2473.6
Abscisic acid	13.9	620.6	634.9	804.2	1001.6	1214.4
Total		3057.9	3100.7	4100.7	5233.0	6171.4

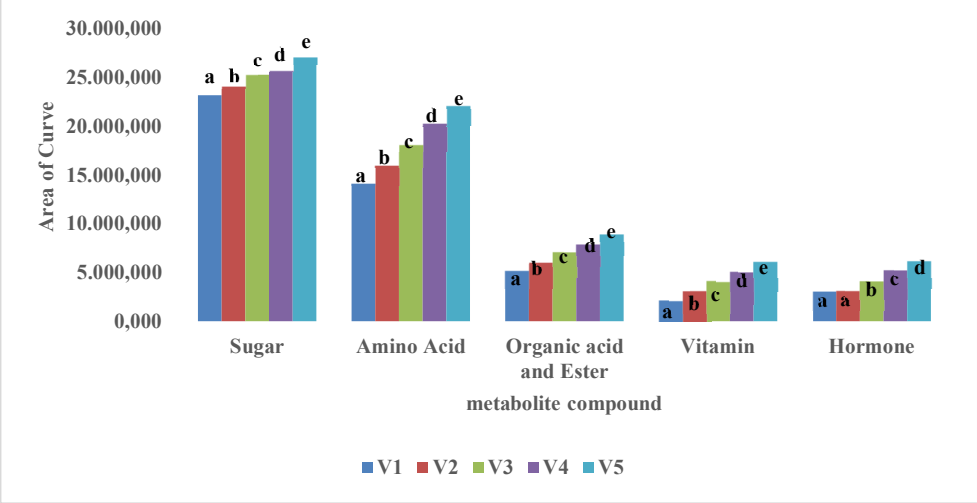

Fig. 2 Graph of metabolites in the LSD test.
Note: different letters in each variable indicate a significance between treatments according to the 5% LSD

B. Discussion

The raw material proportion of 25% soybean husk:75% cow manure (treatment V_5) showed the highest total area of the curve, while the treatment of 100% soybean husk (V_1) had the lowest total area of the curve for all identified metabolites as determined by the LSD test. This indicated that V_5 treatment (25% soybean husk:75% cow manure) was provided the best medium conditions for earthworms to degrade waste. The degradation of waste by earthworms is called vermicomposting, a mesophilic process involving the interaction between earthworms and microorganisms to sequester carbon and degrade cellulose from organic waste into vermicompost [28]–[31]. The earthworm's performance in waste degradation was influenced by its environment (the proportions of soybean husk to cow manure). Earthworms need a relative humidity of 75–90%, temperatures of at least 10 °C, and ideally 15–20 °C, pH 7–9 [13], [32]. These environmental factors were strongly related to the availability of oxygen needed by the earthworms to maintain their activity. High humidity in media with poor aeration will result in the death of the earthworms due to oxygen-starvation and toxic substances such as ammonia produced by various anaerobic microorganisms.

In ideal conditions, earthworms degrade certain substances more rapidly, particularly low-carbon molecules such as sugar. This was demonstrated by the higher number of sugars formed as compared to other compounds. In contrast, complex carbon-containing molecules, such as lignin, not only took longer to break down but also required more enzymes to be broken down. Therefore, in treatment V_1, which was entirely soybean husk, the earthworms needed more energy to degrade the waste; most of the assimilated carbon and energy was diverted to organic molecules synthesis as adaptability against lignocellulosic compounds. The best-performing media met the documented environmental requirements for earthworms and provided adequate nutrients for earthworms and other organisms.

The most-identified compounds in this study were sugars. There were two types of sugar identified in vermicompost, semi-complex sugars and simple sugars. The semi-complex sugars were oligosaccharide sugars consisting of more than two saccharide units, while the simple sugars were either monosaccharide sugars or disaccharide sugars. Compounds included in semi-complex vermicompost sugars, as determined by GC-MS chromatograms, were raffinose and stachyose, while the simple sugar compounds were glucose, glucosamine, maltose, trehalose, and arabinose. Carbohydrates were the most important part of the vermicompost and served as the central component in many biomaterials [33]. They took the form of natural carbonyl compounds of several hydroxy groups (monosaccharide sugars and their polymers, oligosaccharides, and polysaccharides) and were most abundant in living organisms [34]. The function of carbohydrates includes being a source of energy and fuel, an aid to metabolic pathways, a foundation of the structural frameworks of RNA and DNA (ribonucleic acid and deoxyribonucleic acid), and is a structural element in the cell walls of bacteria (peptidoglycan or murein), plants (cellulose), and animals (chitin). Carbohydrates are associated with many proteins and lipids and also have a role in intracellular communication and extracellular interactions [35].

Amino acids are a large group of biomolecules that contain functional groups of amines (-NH2) and carboxyl (-COOH) together with side chains (group R) specific to each amino acid. In plants, amino acids play a role in the central metabolism [36] in all biological systems, as a source of energy as well as the main component in building up cell organelles and enzymes [37],[38] also act as intermediates in both physiological and biochemical metabolic pathways, thereby affecting numerous physiological processes in plants[39],[40]. Organic acids are organic compounds with weak acidic properties and do not fully dissociate in water. The stability of the conjugate base determines its acidity level. Carboxylic acid, with a carboxyl-COOH acidity group is the most common form of organic acid. An example of a strong organic acid is sulfuric acid, which contains –SO2 OH groups. Alcohols with –OH groups can act as acids but are very weak. Organic acids are mainly produced in mitochondria, function as an intermediary in carbon metabolism [41]. In plants, organic acids played a role in the adaptability of plants to nutritional stress conditions [42],[43].

Vitamins are organic molecules (or a collection of related molecules) essential micronutrients or nutrients needed in small amounts for proper metabolism function. Essential nutrition is not something that an organism can synthesize. Each vitamin performs a unique set of reactions and functions, especially is to act as a cofactor in diverse metabolic pathways, facilitate the production of essential compounds for plants and bacteria, induce resistance against pathogens, directly promote plant growth, and participate in energy conversion in the plant from stored compounds [44]. Hormones are signal-carrying chemicals formed in special endocrine glands cells that control cell and plant growth [45]. The most-prevalent hormone in the vermicompost treatments in this study was indole-3-butyric acid (IBA), which belongs to the auxin group, influences root morphology, and is widely applied in various branches of agriculture. [46]–[48].

IV. Conclusions

The potential of soybean husk as a raw material in vermicomposting was successfully evaluated via a metabolomic approach. Treating different soybean husk and cow manure proportions, this study can be used as a guide for soybean husk management alternatives. When combined with cow manure, soybean husk makes it a possible raw material for organic fertilizer. To find out more about the potential of soybean husk as organic fertilizer, more detailed information can be obtained.

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