

## RESEARCH ARTICLE



# The Effectiveness of Bio-enzymes Made from Fruit Waste and Mixture of Fruit Waste and Chicken Intestinal Content as Composting Activators

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## ABSTRACT

The fermentation of fruit waste can produce bio-enzymes. These bio-enzymes may act as composting activators for composting organic materials. This study compared composting activators made from fruit waste, fruit waste enriched with chicken intestinal content, and Effective Microorganism-4 (EM-4) to evaluate their effectiveness for compost production. It was found that the peaks of temperatures of all activators applied were achieved within 3 days, varied between 55–57 °C, and dropped to the lowest point on day 14 at a temperature of 33 °C, indicating that the maturity of the compost fell in the same period. Based on statistical analysis, differences among the three activators were found in the C-organic and moisture content of the compost. However, the compost produced using the three activators, based on the quality standard determined by the Decree of Agriculture Minister of Republic Indonesia No. 261/KPTS/SR.310/6.M/4/2019 and SNI 19-7030-2004 were comparable and met these requirements. In conclusion, composting activators made from fruit waste and fruit waste enriched with chicken intestinal content might be used as composting activators for compost production as effective as composting activator EM-4.

## Introduction

Organic waste accounts for the largest proportion (60%) of all waste generated by human activities [1]. If not handled properly, this organic waste can be a source of groundwater pollution from leachate and air pollution through methane gas production [2]. One type of organic waste is fruit waste, produced in many traditional markets. The most common fruit waste from traditional market activities are bananas, papayas, and pineapples, which have not been utilized to produce useful products. Recycling and reusing fruit waste can produce bio-enzymes that have multipurpose uses and are eco-friendly [3,4]. The fermentation of fruit waste can obtain bio-enzymes. The bio-enzymes produced can vary depending on the microbes' diversity in the fermentation process. Chin et al. [5] identified high enzyme activity from protease,  $\alpha$ -amylase, and cellulase. The many enzymes produced from the fermentation process make fruit bio-enzymes usable for various uses, one of which is a composting activator [5,6].

The characteristics of fruit bio-enzymes are influenced by the type of fruit waste used, local microorganisms involved in the fermentation process, and ingredients such as chicken intestinal content in bio-enzymes production. Adding intestinal contents is intended to enrich the diversity of the microbes involved in fermentation. Using local microorganisms is beneficial because they have already been adapted to local conditions. If bio-enzymes, as composting activators that contain local microorganisms, are as effective as commercially known composting activators, this will benefit local farmers. Local farmers can produce their composting activator and will not depend on commercial products, such as EM-4, which are often unavailable and difficult to afford in remote areas. Therefore, this study aimed to assess the effectiveness of fruit bio-

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enzymes containing local microorganisms as composting activators by comparing them to the commercial composting activator product of EM-4.

## Materials and Methods

### Study Area

Bio-enzyme production and testing of their effectiveness as activators in composting organic materials were conducted at *Kesatuan Pengelolaan Hutan Lindung* (KPHL) Biak Numfor, Papua Province, Indonesia. Pineapple, papaya, and banana fruit waste were obtained from a traditional market in Biak City, whereas chicken intestinal content was obtained from local chicken breeders in Biak city. The air temperature at the time of testing ranged from 30 °C to 33.5 °C, with humidity ranging from 79% to 90%.

### Materials

This study used three bio-enzymes as composting activators: bio-enzymes made from fruit waste, bio-enzymes made from fruit waste enriched with chicken intestinal content, and Effective Microba-4 (EM-4). Bio-enzymes are made from fruit waste, containing a mixture of overripe bananas, pineapples, and papaya. The Pisang raja variety was used as a banana. EM-4 is a solution that contains microorganisms resulting from fermentation, is known as a composting activator, and is commercially available on the market. The other materials used for bio-enzyme preparation were molasses made from sugar (sucrose). The compost was prepared using the same basic ingredients, namely dried goat manure, sawdust, rice bran, and *Gliricidia sepium* leaves. Sugar (sucrose) was added to the composting activator solution. Composting was performed using a plastic container with a lid as a bioreactor, with a volume of 50 L, diameter of 50 cm, and height of 50 cm.

### Experimental Design

The assessment was conducted using a completely randomized design. Three composting activators were used as treatments: (1) BF = bio-enzyme made from fruit waste; (2) BC = bio-enzyme made from fruit waste enriched with chicken intestinal content; and (3) BE = bio-composting of Effective Microba-4 (EM-4). All the treatments were performed in five replicates. Variables measured to monitor the composting were pH, temperature, and changes in compost weight, while chemical and physical characters used were C organic variables (%), total P<sub>2</sub>O<sub>5</sub> (%), total K<sub>2</sub>O<sub>5</sub> (%), Ca<sup>++</sup> (%), Mg<sup>++</sup> (%), Na<sup>++</sup> (%), moisture content (%), color, odor, the texture of the compost and the mass reduction.

The temperature and pH of the compost were monitored and measured daily. Thermometers were inserted into the compost, and data were recorded. pH (H<sub>2</sub>O) of the compost was determined using a potentiometer. The change in the weight of the compost was determined by comparing the initial compost weight with the final weight of fresh compost produced after the compost was ripe. The total compost production was measured by weighing the compost after air-drying for a week. The moisture content was measured using a gravimetric method. Odor performance was examined by smelling, the color of the compost was determined using a Munsel color chart, and the texture of the compost was determined by the felling method.

Bulk samples of compost were collected at the end of the experiment using a simple random sampling method to obtain representative samples from each experimental unit. Bulk samples were used for chemical analysis of organic matter content, total N, C/N ratio, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O<sub>5</sub>, Ca<sup>++</sup>, Mg<sup>++</sup>, and Na<sup>++</sup>. Organic matter content was determined using the Walkley-Black method, total nitrogen by the Kjeldahl method, P<sub>2</sub>O<sub>5</sub> by the spectrometric method, K<sub>2</sub>O<sub>5</sub> by the Photometric method, and Ca<sup>++</sup>, Mg<sup>++</sup>, and Na<sup>++</sup> by the atomic absorption method.

### Bio-enzyme Preparation

Banana, pineapple, and papaya waste with the same weight proportion (w/w, 1:1:1) were cut into small pieces. Molasse is made by dissolving palm sugar (1,000 g) in boiling water (1,000 ml) and stirring until completely dissolved. Furthermore, all ingredients in the form of fruit waste (3 kg), water (1 L), and molasses (1 L) were evenly mixed in a covered plastic container as a reactor for fermentation. The fermentation reactor was placed in a cool place and not exposed to sunlight. Fermentation was performed within 14 days. The fruit bio-enzyme (BF) was then filtered to obtain a solution. While the production of fruit bio-enzyme + chicken intestinal content (BC) was made with the same composition as BF, the difference was only in adding 200 mg of the chicken intestinal content.

## Composting

The basic compost materials consisted of dried goat manure (6 kg), sawdust (1.5 kg), rice bran (1 kg), and fresh *Gliricidia sepium* leaves (1.5 kg). The composting activator was prepared by mixing 1 ml of bio-enzyme, 2 g of sugar (sucrose), and 1 L of clean water. The composting activator solution was incubated for 1 h before use. All the basic compost materials were then placed in a plastic container as a bioreactor and mixed evenly. The compost mixture was treated with a composting activator. Each experimental unit was given a composting activator (BF, BC, or BE), according to the treatment design. This process was performed slowly until the composting activator solution completely wetted all parts of the compost material. Ensuring that the given composting activator is sufficient can be achieved by clenching compost material. A composting activator solution was considered sufficient if the compost did not release water when it was clenched, and if the clenching was released, the compost material expanded again. The compost material, readily applied as a composting activator solution, was then incubated. The daily temperature and pH of the compost were measured to determine the end of the experiment.

## Data Analysis

Analysis of Variance (ANOVA) was performed to evaluate the variation caused by the composting activator treatments on the compost characteristics. Statistical analysis using the mean difference test following the Honest Significant difference (HSD) was performed if ANOVA showed differences given by the composting activator treatments. Differences in pH and temperature at the end of the experiment caused by the composting activator treatments were analyzed using a paired t-test. The quality of the compost produced was evaluated following SNI 19-9030-2004 [7] and the Decree of the Agriculture Minister of the Republic of Indonesia No. 261/KPTS/SR.310/6.M/4/2019 [8].

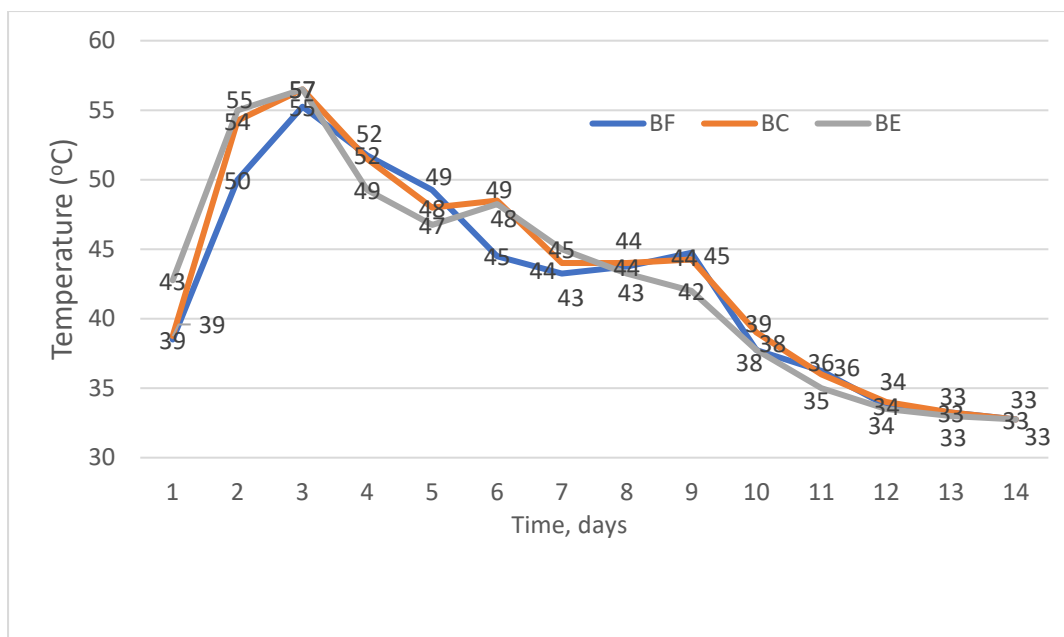
## Results and Discussion

### Temperature and pH Dynamic During Composting

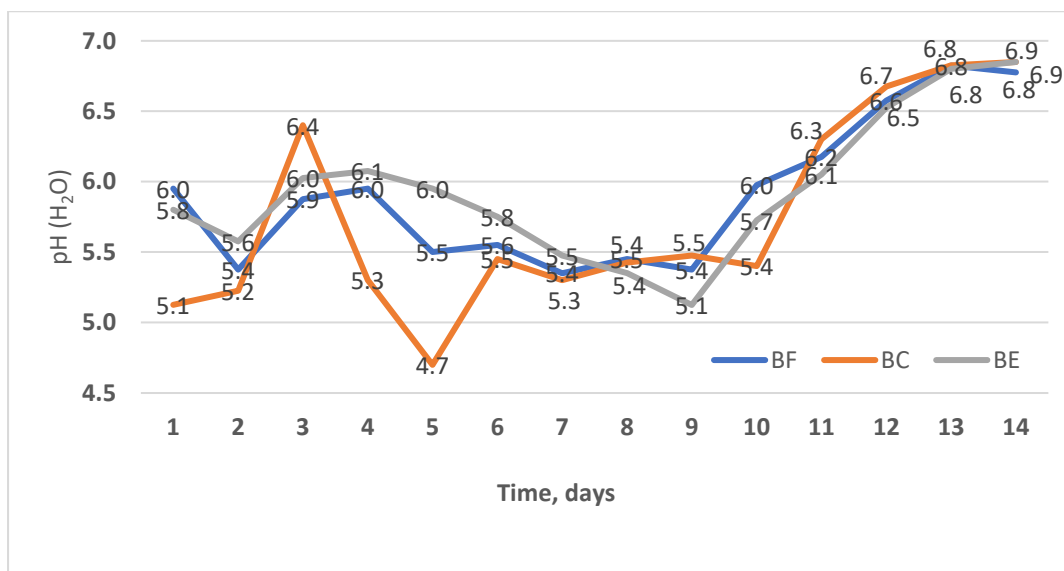
Composting was performed for 14 days using BF, BC, and BE activators. During composting, the temperature and pH of the compost material exhibited similar fluctuating patterns (Figure 1). The paired t-test results supported the similarity of the dynamic patterns of temperature and pH of the compost from the three composting activator treatments. There was no difference in temperature and pH between the treatments at the 95% confidence level (Table 1). The initial temperature of the compost material among the treatments, when incubation began, was similar. The temperatures of BF and BC were the same (39 °C), whereas the BE was slightly higher (43 °C). The peak temperatures achieved on day 3 were 57 °C for BC and BE, and 55 °C for BF.

After the temperature peaked, it gradually decreased, and the lowest temperature during the incubation period of the compost materials occurred on day 14. At this point, all three treatments had the same temperature of the compost materials (33 °C), and the acidities of the three composts (BF, BC, and BE) did not show a significant difference and were in the pH range of 6.8–6.9. The materials used for compost production were identical among the treatments, so the abundance and variety of microbes would determine the course of the composting process and the quality of the compost produced. The temperature of compost can be used as an indicator of compost maturity [9]. An elevated compost temperature reveals that the metabolic activity of mesophilic microbes increases, indicating that the decomposition of the compost constituents is occurring [10,11].

The temperature peaks in the BF and BC treatments reached 57 °C, whereas the BE reached 55 °C, and there were no differences between them according to the t-test (Table 1). These temperatures achieved the thermophilic temperature, which is in the range of 40 to 65 °C [12,13]. Pathogenic microorganisms are inactivated by achieving a thermophilic temperature >70 °C [14]. The compost temperature significantly governs the activity of the decomposer and biomaterial decomposition during composting [15,16]. The composting process consisted of three stages: mesophilic, thermophilic, and cooling or maturation. During the mesophilic stage, the compost temperature rises to reach the temperature of about 40 °C; in this stage, mesophilic microbes dominate. Gradually, thermophilic bacteria, fungi, and actinomycetes are replaced as the temperature increases between 50 and 60 °C [17,18].



(a)



(b)

**Figure 1.** Changes in temperature (a), and pH (b) of compost material during the composting process using fruit bio-enzyme (BF), fruit + chicken intestinal content bio-enzyme (BC), and EM4 (BE) as composting activators.

**Table 1.** Differences in temperature and pH of compost during the 14-day incubation period due to BF, BE, and BC treatments.

Variables	T Values	p-Values*
Temperature (BF) vs Temperature (BC)	-1.68	0.117 <sup>ns</sup>
Temperature (BF) vs Temperature (BE)	-1.65	0.528 <sup>ns</sup>
Temperature (BC) vs Temperature (BE)	-1.65	0.525 <sup>ns</sup>
pH (BF) vs pH (BC)	-1.70	0.113 <sup>ns</sup>
pH (BF) vs pH (BE)	-0.25	0.810 <sup>ns</sup>
pH (BC) vs pH (BE)	-1.55	0.146 <sup>ns</sup>

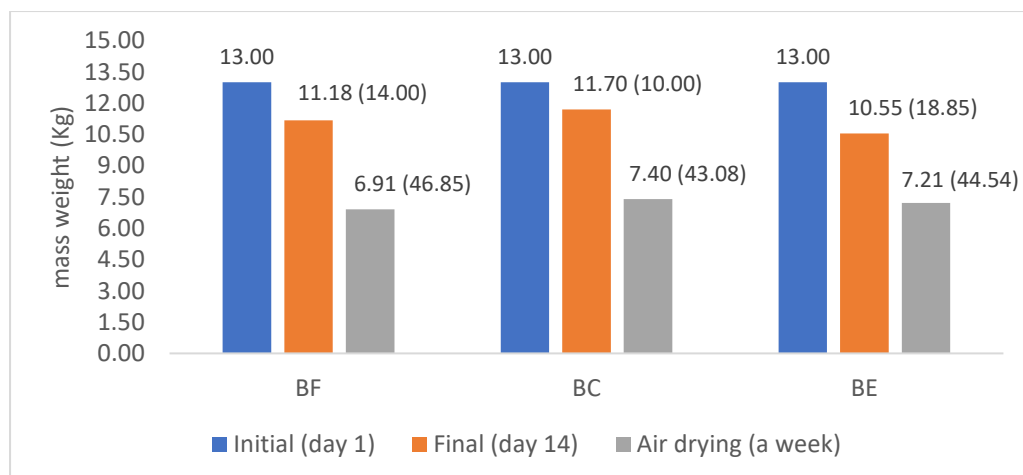
\* ns = not significant; s = significant on the confidence interval of 95%.

According to Rynk et al. [19], temperatures ranging from 30 to 60 °C indicate fast composting activity. After the temperature peaked, it decreased to the lowest temperature point, and the temperature was relatively stable, which in this study was achieved on day 14 at a temperature of 33°C in all composting activator treatments. A fading smell of ammonia will also follow this decrease until it finally turns into an earthy smell. Such conditions indicate that all of the organic matter composed of it has been completely decomposed, which causes a decrease in microbial activity to the lowest point, indicating that the compost is ready to be harvested.

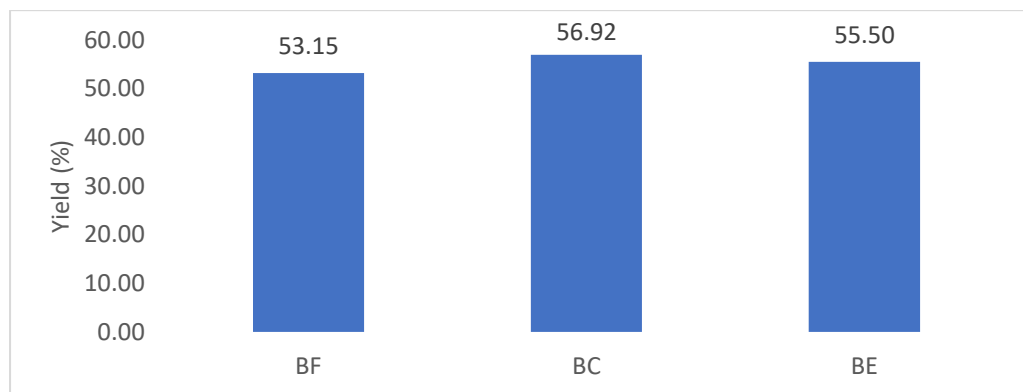
The initial pH values were slightly different among the treatments. The highest initial pH of the compost materials was found in BF (pH 6.0), followed by BE (pH 5.8), and BC (pH 5.1), and there were no differences according to the t-test (Table 1). The decrease in pH may be related to the presence of lactic acid bacteria and sufficient ready-to-ferment sugars, which produce lactic acid and other organic acids as well as carbon dioxide and alcohol when the composting reactor conditions lack oxygen [20,21]. This fluctuating temperature trend was in contrast to the compost pH pattern. When the decomposition activity increased, the compost temperature increased, whereas the pH of the compost decreased, which is in line with the increase in the production of organic acids. The end of composting was indicated by a decrease in microbial activity, as was evident from the temperature decline and pH increase. The pH reached 6.8 in the BF and BC treatments and 6.9 in the BE treatment. These pH values are within the requirements of the SNI 19-7030-2004 standard (pH 6.8 to 7.49) [7].

### The Weight Lost During Composting

The initial compost weight was the same (13 kg). After 14 days of incubation, the highest weight loss occurred in BE (18.85% reduction), followed by BF (14% reduction) and BC (10.0% reduction). Drying the compost for one week in the sun resulted in a reduction of 46.85% in compost weight in BF, 44.54% in BE, and 43.08% in BC. The yields obtained were 53.15% for BF, 55.46% for BE, and 56.92% for BC (Figure 2).



(a)



(b)

**Figure 1.** Weight loss of compost material during 14 days of incubation, a week of sun drying (a), and compost yield after sun drying for a week (b); The values in parentheses represent the weight loss of the compost material (%).

The decrease in compost material weight demonstrated the effectiveness of the composting activator. The weight loss of compost is related to the loss of water from the compost materials. The presence of complex compounds such as lignin, which are difficult to degrade, causes a small decrease in the weight of compost material biomass [12]. The weight loss of compost may be due to the high consumption of H<sub>2</sub>O during microbial metabolic activities and some loss through evaporation resulting from the high temperature during the thermophilic period. The compost shrinkage achieved after 14 d of incubation was 18.85% for BE treatment, 14.00% for BC, and 10.00% for BF. After air drying, the shrinkage at BF reached 46.85%, followed by BE at 44.54%, and BC at 43.08%. This is comparable to the weight loss during composting of as much as 40 to 50% of the initial weight of wheat straw as compost material [22]. Compost air drying causes the removal of water from the compost and, in turn, results in loss of compost mass. After air-drying, from the total initial weight of organic materials, 56.92% compost in BC, 55.50% in BE, and 53.14% in BF.

## Chemical and Physical Characteristics of the Composts

### Chemical Characteristics of the Composts

ANOVA revealed that the treatments only affected the compost's C-organic and moisture content at the experiment's end. Even though there were differences in these two variables, the composts produced using the different composting activators still followed the standard quality based on the minimum requirements for solid organic fertilizer Republic of Indonesia Decree No. 261/KPTS/SR.310/6.M/4/2019 [8], and SNI 19-7030-2004 [7], concerning the specifications for compost from domestic organic waste (Table 2). The nutrient content (N, P, K, Mg, and Ca) in the compost produced on day 14 was comparable among the treatments. Thus, the use of BF and BC was comparably effective as BE composting activators, which are widely known and commercially available.

**Table 2.** Chemical and physical characteristics and the quality of composts produced through the application of bio-enzymes as composting activators.

No.	Compost characteristics	Composting activators			Quality standard
		BF	BC	BE	
1.	C-organic (%)	38.05 a <sup>#</sup>	37.58 ab	36.75 b	≥15% <sup>1)</sup> 9.30–32% <sup>2)</sup>
2.	Total N (%)	1.76 a	1.77 a	1.82 a	≥ 0.50% <sup>1)</sup> ≥ 0.40% <sup>2)</sup>
3.	C/N Ratio	22.00 a	21.55 a	20.00 a	≤ 25 <sup>1)</sup> 10-20% <sup>2)</sup>
4.	Total P <sub>2</sub> O <sub>5</sub> (%)	1.79 a	1.80 a	1.90 a	N+P <sub>2</sub> O <sub>5</sub> +K <sub>2</sub> O ≥ 2% <sup>1)</sup> ≥ 0.10% <sup>2)</sup>
5.	Total K <sub>2</sub> O (%)	0.88 a	0.81 a	0.84 a	N+P <sub>2</sub> O <sub>5</sub> +K <sub>2</sub> O ≥ 2% <sup>1)</sup> ≥ 0.20% <sup>2)</sup>
6.	Ca <sup>++</sup> (%)	8.58 a	9.08 a	8.92 a	25.5% <sup>3)</sup>
7.	Mg <sup>++</sup> (%)	0.99 a	1.04 a	1.09 a	0.6 % <sup>3)</sup>
8.	Na <sup>+</sup> (%)	0.57 a	0.53 a	0.53 a	≤ 2000 ppm <sup>1)</sup>
9.	Moisture Content (%)	36.28 a	29.47 b	30.15 b	10–25% <sup>1)</sup> ≤ 50 % <sup>2)</sup>
10.	pH (H <sub>2</sub> O)	6.8 a	6.8 a	6.9 a	4.0-9.0 <sup>1)</sup> 6.80-7.49 <sup>2)</sup>
11.	Color <sup>4)</sup>	dark brown, 10YR 4/3	dark brown 10YR 4/1	dark brown 10YR 4/2	dark brown
12.	Odor	Soil odor	Soil odor	Soil odor	Soil odor <sup>2)</sup>
11.	Texture	Crumb	crumb	crumb	- <sup>3)</sup>

<sup>#</sup> Numbers followed by the same letters in one row were not significantly different based on Tukey Mean Test (p<0,05). <sup>1)</sup>The minimum requirements for solid organic fertilizers are based on the Decree of the Agriculture Ministry of the Republic of Indonesia No.261/KPTS/SR.310/6.M/4/ 2019 [8]; <sup>2)</sup>Compost quality standards based on SNI 19-7030-2004 [7]; <sup>3)</sup>Does not include the scope of the assessment of the minimum requirements for organic fertilizers; <sup>4)</sup>Munsell soil color chart.

The length of time to reach compost maturity is related to the rate of degradation of the organic matter making up the compost, which is influenced by aeration, moisture conditions, pH, temperature, relative abundance of microbes, and content of easily degradable constituents. The decomposability of organic materials depends on their nitrogen content with protein-rich substrates. Microbes can easily metabolize organic materials. Compost materials with a high C/N ratio often contain complex organic compounds, such

as tannins, that inhibit decomposition. Therefore, the C/N ratio is often used to measure decomposability [17,23–25]. In addition, Partanen et al. [20] stated that there is a close relationship between the composition dynamics of the bacterial community and changes in the chemical and physical properties of the compost material degraded during the decomposition process.

The C/N ratio decreased as the compost material decomposed [26]. The decrease in the C/N value is due to microbes using carbon as an energy source and lost in the form of carbon dioxide through respiration. An increase in nitrogen accompanies this decrease in carbon [11]. The C/N ratios of the three composting activator treatments were similar: 22.0%, 21.5 %, and 20.0% in BF, BC, and BE, respectively. A C/N ratio close to 20 is said to be phytotoxic-free, even though the compost is not yet ripe [12,13]. However, based on statistical tests, there was no significant difference in the C-organic content on day 14 between BE (36.75%) and BF (38.05%) or between BC (37.58%) and BE (36.75%), but there was a difference between BF and BE. This shows a relatively better degree of organic matter degradation in the BE.

#### ***Compost Odor***

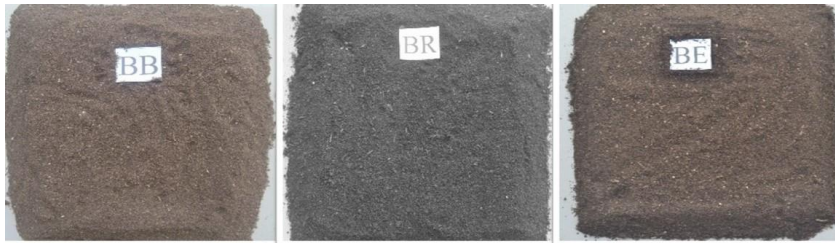
Initially, the compost had a dominant smell coming from the compost ingredients; then, it changed to the smell of ammonia when the compost temperature peaked and slowly faded to an earthy smell on day 14. There was no difference in smell between the composting activator treatments. An increase in temperature is accompanied by an increase in the unpleasant odor of ammonia, resulting from the decomposition of nitrogen in the organic matter complex, which is converted into ammonia through ammonification. When oxygen is available, nitrification occurs, and nitrifying bacteria oxidize ammonia into nitrate [27]. H<sub>2</sub>S is produced under anaerobic [28]. Fauzan et al. [29] added that a high C/N ratio of the raw materials plays a role in unpleasant odor production because perfect decomposition does not occur. Lalremruati and Devi [17] stated that materials with a very low C/N ratio may also lead to odor problems. The material contained too much N, which likely released ammonium gas into the atmosphere. Steger et al. [30] added that adequate composting may occur at O<sub>2</sub> levels as low as 2.5 and 1%, but this is not recommended because anaerobic conditions might be present, which is not ideal for efficient composting.

#### ***Compost Moisture***

The fresh organic materials used as the basic ingredients for compost resulted in the initial moisture content of the compost being relatively high, reaching 60 to 70%, which then decreased in line with the decomposition process. When decomposing organic matter, complex compounds, such as starch, protein, hemicellulose, cellulose, and lignin, are broken down into simpler ones requiring a large amount of water. However, when microbial metabolic activity increases and high heat is generated, a large amount of water is lost via evaporation [10,31]. At the end of the composting period, different compost moisture contents were observed in the BF (36.28%), BC (29.47%), and BE (30.15%) treatments. This difference may be due to variations in microbial metabolic activity, as indicated by the highest temperature achieved by the BC and BE treatments (57 °C) compared to the BF treatment (55 °C). Even so, the compost produced by all treatments met the moisture content standards of SNI 19-7030-2004 ( $\leq 50\%$ ) [7].

#### ***Compost Color***

Likewise, the compost produced by the composting activators had a blackish brown color, although the BF and BE treatments were lighter than the compost in BC (Figure 3). Color changes during composting can be used to measure the compost maturity. The color change also indicates the level of stabilization achieved by the compost [12]. A change in color to black indicated that the compost had reached maturity [31]. The color of the compost is influenced by the composition of the compost material, which is decomposed owing to the presence of dissolved organic matter particulates [12]. Materials with a high C/N ratio, such as sawdust, cause the brown color of the compost. The use of raw materials for compost production in this study was the same among all treatments. Therefore, the color variation among the treatments is predictably related to the different degrees of decomposition resulting from the different amounts of dissolved organic matter particulates.



**Figure 3.** The colors of the final product of composting using fruit bio-enzyme (BF), fruit + chicken intestinal content bio-enzyme (BC), and EM4 (BE) as composting activators.

## Conclusions

Composting organic material consisting of dry goat manure (6 kg), sawdust (1.5 kg), rice bran (1 kg), and fresh leaves of *Gliricidia sepium* (1.5 kg) required 14 days to obtain compost with perfect maturity using composting activators made from fruit waste (BF), fruit waste + chicken intestinal content (BC), and EM4 (BE). The highest composting temperature was achieved on day 3 at 55 °C for BF and 57 °C for BC and BE. The physical and chemical characteristics of the compost produced using BF, BC, and BE were in the range of quality standards of compost production applicable in Indonesia. The nutrient contents of the compost produced by BF and BC were comparable to that produced by BE. Compost yield was achieved in the range of 53.15 to 56.92%. Based on this assessment, composting activators made from fruit waste (BF) and waste plus chicken intestinal content (BC) may become an alternative substitute for EM4 (BE) for compost production. Searching for the basic composition of compost by increasing the amount of sawdust to the optimal level to produce compost with acceptable quality organic fertilizer standards needs to be done.

## Author Contributions

**MSM:** Conceptualization, Methodology, Investigation, Writing - Original draft & Editing; **JDN:** Conceptualization, Methodology, Formal analysis, Writing - Review & Editing, Supervision; **MM:** Conceptualization, Methodology, Writing - Review & Editing, Supervision.

## Conflicts of interest

There are no conflicts of interest.

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