Research Article

Enhancement of Black Tea Aroma by Adding the β-Glucosidase Enzyme during Fermentation on Black Tea Processing

Supriyadi Supriyadi1, Alfrista Ruri Nareswari,1 Aprilia Fitriani2, and Rachmad Gunadi3

1Department of Food and Agriculture Products Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
2Food Technology, Faculty of Life Science, Surya University, M.H. Thamrin Street KM 2.7 Grand Serpong Mall 1st Floor F8 and F9 Units, North Panunggangan, Pinang, Tangerang City, Banten 15143, Indonesia
3Department of Soil Science, Faculty of Agriculture, Gadjah Mada University, Yogyakarta, Indonesia

Correspondence should be addressed to Supriyadi Supriyadi; suprif248@ugm.ac.id

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Black tea aroma is one of the essential attributes in determining the quality of black tea. β-Glucosidases were investigated for their ability to enhance the aroma of black tea by hydrolyzing the glycoside compound. The addition of β-glucosidase was done by dissolving the enzyme on a sodium citrate buffer (pH 5.0), which was then sprayed on tea leaves during black tea processing. The β-glucosidase treatment significantly increases the volatile compound from glycoside precursors such as linalool, geraniol, and methyl salicylate. Moreover, the volatile compound from carotenoid and lipid precursors (nerolidol and β-cyclocitrinal) was also increased with β-glucosidase treatment.

1. Introduction

Tea aroma is one of the essential properties which are decisive for the quality of the final product. The tea product aroma can be formed through various precursors such as carotenoids, fatty acids, terpenoids, phenylpropanoid/benzenoid, glycoside hydrolysis, and the Maillard reaction [1]. In black tea, aroma formation occurs due to oxidation and degradation of fatty acids and hydrolysis of glycosides [2]. cis-Jasmone and methyl jasmonate, which are derived from fatty acids, have fresh floral odors. But most of the other compounds derived from fatty acids are unpleasant, such as (Z)-2-hexenal and (Z)-3-hexenal, which have a grassy odor, and (E,E)-2,4-heptadienal and 2-butanone with a fatty aroma [3]. Apart from the oxidation and degradation of fatty acids, the formation of aroma in black tea also occurs due to glycoside hydrolysis. The injured tea leaf tissue will release enzymes into the cell walls to hydrolyze the glycosidic bond and release volatile compounds [4]. Most of the volatile compounds formed by glycoside hydrolysis have a pleasant aroma, such as methyl salicylate (fresh, sweet), β-damascenone (sweet, rose-like), and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (caramel-like, sweet).

β-Glucosidase is one of the glycosidase enzymes that can hydrolyze glycosidic bonds [5]. The β-glucosidase enzyme could hydrolyze glycosides and release various free volatile compounds such as linalool and geraniol [6]. These compounds have a fruity floral odor and contribute significantly to black tea [7]. However, the endogenous enzyme β-glucosidase has not optimally hydrolyzed all of the glycosides present in tea leaves. Glycosides such as benzyl β-D-glucopyranoside, 2-phenylethyl β-D-glucopyranoside, (Z)-3-hexenyl-β-D-glucopyranoside, geranyl β-D-glucopyranoside, and linalyl β-D-glucopyranoside are still detectable in black tea products. Thus, the addition of the β-glucosidase enzyme in the black tea processing needs to be done to maximize the hydrolysis of glycosides in tea leaves to produce black tea products with a better aroma.
The enzyme β-glucosidase is widely used in fermented beverage products such as wine, oolong tea, and black tea. The β-glucosidase enzyme in black tea infusion increased the concentration of linalool and geraniol compounds by 10% and 18.5%, respectively [8]. The concentration of methyl salicylate and Z-3-hexenol in steeping black tea increased by 2.14% and 8.24%, respectively, with the addition of the β-glucosidase enzyme [9]. However, it is unclear how β-glucosidase could improve the aroma of black tea during processing. Therefore, the effect of adding the β-glucosidase enzyme, especially on aroma and other chemical components, during the black tea processing needs to be studied further.

2. Materials and Methods

2.1. Materials. Fresh tea leaves (PGL 15 clones) with shoot tips and two leaves underneath (p+2) were plucked in August 2020 from block Sanderan (1000 above sea level) in the Pagi-laran plantation site, Batang, Central Java. The β-glucosidase enzyme (400 U/gr) was from Xi’an Geekee Biotech (China); p-nitrophenyl-β-D-glucopyranoside (p-NPG) and p-nitrophenol (pNP) were from Shanghai Kayi Chemical (China); the other materials used are as follows: acetone (Merck), polyvinylpolypyrrolidone (PVPP) (Merck), sodium carbonate, Folin-Ciocalteu reagent, catechol (Merck), oxalic acid, sodium hydrogen carbonate, and ethyl acetate.

2.2. Methods

2.2.1. Preparation of Acetone Powder. The preparation of acetone powder is based on a previous study [10]. Around 20 gr fresh tea leaves were homogenized with cold acetone (400 mL; -20°C), and the suspension was filtered on a vacuum filter (Whatman filter paper no. 44) and then washed repeatedly three more times with cold acetone (200 mL) until colorless. Finally, the resultant acetone powder was dried in a desiccator. The dried powder was stored at -20°C.

2.2.2. Preparation of Crude Enzyme Solution. The crude enzyme solution was prepared with 0.8 gr acetone powder, 0.4 gr PVPP, and 20 mL of a 50 mM sodium citrate buffer (pH 5.0). The mixture was centrifuged at 10000g for 25 min at 4°C. The supernatant was used as the crude enzyme solution for the assay of β-glucosidase and polyphenol oxidase activity [10].

2.2.3. Assay of β-Glucosidase Activity. A substrate (p-NP-β-D-glucopyranoside) was prepared in a 50 mM sodium citrate buffer (pH 5.0). A 200 μL substrate was incubated at 37°C for 5 min; then, 100 μL of the enzyme was added, and the reaction mixture was incubated at 37°C for 15 min. The reaction was stopped by adding 1.4 mL of sodium carbonate solution (0.2 M). The resulting yellow color was measured at 420 nm by a spectrophotometer using p-nitrophenol as the standard. One unit of β-glucosidase activity is defined as the amount of enzymes that hydrolyzed 1 μmol of the substrate/min at 37°C [10].

2.2.4. Preparation of Black Tea. Black tea was made manually; fresh tea leaves (PGL 15 clones) that have been plucked were immediately subjected to the withering process using a withering machine in the factory. The process took about 12-15 hours. The following method was rolling and crushing tea shoots manually; after obtaining rolled and smaller tea leaf shoots, they are left for 2 hours at room temperature with periodic reversals. This process is also called enzymatic oxidation. The β-glucosidase enzyme (25 mg/10 mL) was added at the beginning of the enzymatic oxidation process. After 2 hours, the enzymatic oxidation process (fermentation) was immediately stopped by pan frying or roasting at 100 ± 5°C for 10 minutes. The final process was sieving the dry black tea to obtain the uniform size.

2.2.5. Identification of Volatile Compounds in Black Tea. Based on the previous study, identification of aroma compounds in black tea was carried out [11] with modifications. Briefly, 2 gr of the ground dried black tea sample and 0.5 μL of ethyl decanoate (as the internal standard (IS)) were mixed in a 22 mL vial. Subsequently, the vial was placed in an oven at 80°C for 15 min to balance the headspace gas. Afterward, a 50/30 μmdivinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 2 cm fiber was inserted into the headspace vial, and the volatiles were absorbed for 30 min under an 80°C oven condition. Volatile compounds of black tea samples were analyzed using the GC Agilent 7890A instrument with an MS Agilent 5975 XL EI/CI detector. Helium gas is carrier gas with a flow rate of 0.8 mL/min. Desorption of volatiles from samples was accomplished by inserting the SPME needle for 10 minutes at the injection temperature of 250°C, and the splitless method was used in this analysis. The oven temperature was set in a gradient with the initial temperature setting of 50°C held for 3 minutes, then increased to 190°C at a rate of 5°C/min, then improved to 220°C at a rate of 4°C/min, and held for 3 min. The mass scan range was set to m/z 29-550. The GC-MS instrument was coupled with a mass spectrometer detector with an HP-5 capillary column (Agilent, USA; 30 m, inner diameter 0.25 mm, and layer thickness 0.25 μm). Chromatograms were evaluated with Xcalibur software version 1.4 (Thermo Fisher Scientific), and mass spectra were matched with NIST Library version 2005. All experiments were repeated three times. Component identification used mass spectral references in the NIST Library 2005 and retention indices (RI) [12]. RI was calculated using standard alkanes (C9-C20) under the same conditions as the sample analysis conditions.

3. Result and Discussion

3.1. The Activity of the β-Glucosidase Enzyme during Black Tea Processing. Most enzymes are proteins that can accelerate or catalyze a chemical reaction and produce a product. The activity of an enzyme is closely related to the amount of products made in units of time. The activity of the β-glucosidase enzyme during the black tea processing can be seen in Figure 1.

Figure 1 shows that the β-glucosidase enzyme activity in PGL 15 fresh leaves (P15) was 0.80 ± 0.03 μmol/min/gr acetone powder and increased significantly at the withering stage (P15 L) to 0.93 ± 0.05 μmol/min/gr acetone powder.
Compared to the P15 L, β-glucosidase enzyme activity in factory withered leaves (DLP) was not significantly different, namely, 0.91 ± 0.05 μmol/min/gr acetone powder. The same condition also occurred in the previous study [10], where the β-glucosidase enzyme activity was highest during withering. In this study, the withering stage was carried out for 15 hours. The increased concentration of cell fluids during the withering process also increased enzyme concentration, resulting in improved enzyme activity [13]. At the fermentation stage, the activity of the β-glucosidase enzyme in the sample of PGL 15 (P15 F) and PGL 15 leaves with β-glucosidase enzyme treatment (P15+e F) decreased significantly to 0.33 ± 0.04 μmol/min/gr acetone powder and 0.46 ± 0.03 μmol/min/gr acetone powder. The same thing also happened in the fermentation of the leaves from the factory (DPF), which was 0.35 ± 0.04 μmol/min/gr acetone powder. This decrease in enzyme activity is probably due to the interaction between the enzyme and the substrate. So, the amount of substrates decreases and results in reduced activity of the β-glucosidase enzyme. This result is supported by the number of substrates (glycoside compounds) significantly reduced at the fermentation stage [14, 15].

At the fermentation stage, PGL 15 clones treated with the β-glucosidase enzyme (P15+e F) had a higher amount of enzyme activity compared to PGL 15 clones without enzyme treatment (P15 F). This phenomenon can occur because the enzyme is still able to react with the remaining substrates. The endogenous enzymes in tea leaves could not hydrolyze the substrate completely during the tea processing [16]. So more substrates will be hydrolyzed by adding the β-glucosidase enzyme.

### 3.2. Effect of the β-Glucosidase Enzyme on Glycosidic Bound Volatile Compounds

The addition of the β-glucosidase enzyme is often used to increase the aroma in fermented beverages such as wine and black tea. The effect of adding the β-glucosidase enzyme in making black tea can be seen in Table 1.

As many as 13 volatile compounds from the hydrolysis of glycosides were identified in the black tea sample. As a comparison, volatile compounds in black tea from the processing factory of PT Pagilaran were identified. Several volatile compounds were detected. 3-Hexenol is one of the volatile compounds derived from the hydrolysis of the glycosidic bonds in glycosides. This compound is a joint compound found in tea and has a green fresh odor. The volatile 3-hexenol compound is released from glycoside (Z)-3-hexenyl-β-D-glucopyranoside, hydrolyzed by the β-glucosidase enzyme, leaving β-D-glucopyranoside as residual sugar [6, 17]. In the THE sample, as much as 11.60 ppb was detected, twice as much as that of the THK sample detected at 5.31 ppb. The result shows that the addition of the β-glucosidase enzyme can hydrolyze more glycosides. In comparison, the factory black tea was seen to be as much as 19.70 ppb. As discussed by Ni et al., 3-hexenol can be isomerized to 2-hexenol [17], but this compound was not detected in the black tea sample in this study.

Other alcohol compounds detected in the black tea sample were benzyl alcohol and phenylethyl alcohol. Ho et al. explained that phenylethyl alcohol and benzyl alcohol are volatile compounds released due to the hydrolysis of the glycosidic bonds [4]. Phenylethyl alcohol is produced from the hydrolysis of phenylethyl-β-D-glucopyranoside and has a floral rose odor. Phenylethyl alcohol has a higher concentration in black tea with the addition of β-glucosidase enzyme (THE), namely, 18.40 ppb, compared to without the enzyme (THK), which is 12.97 ppb.

Benzyl alcohol is also a volatile compound from glycoside precursors. Benzyl alcohol is released from hydrolysis of the benzyl β-D-glucoside compound by the β-glucosidase enzyme and has a floral rose odor [6]. However, this study shows that the benzyl alcohol in black tea with the addition of the β-glucosidase enzyme (THE) has a lower concentration when compared to THK. This result may occur because the addition of the β-glucosidase enzyme can accelerate the enzymatic oxidation reaction so that many benzyl alcohol compounds have been converted into benzaldehyde [18].

Hydrolysis of glycosides released volatile monoterpene alcohol compounds such as geraniol (sweet, honey-like), linalool (floral), and linalool oxide (sweet, floral, creamy) [4, 19, 20]. In this study, the monoterpene alcohol compounds detected were linalool, linalool oxide, trans-linalool oxide, cis-linalool oxide, epoxylinalool, geraniol, and cis-geraniol.

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**Figure 1:** Changes of β-glucosidase during the black tea processing. DS: fresh leaves; DL: withered leaves; DLP: factory withered leaves; DF: fermented leaves; DF+e: fermented leaves with the β-glucosidase enzyme; DFP: fermented factory leaves. The error bars represent the standard deviation of measurements for five samples.
These compounds have higher concentrations in black tea with the addition of the β-glucosidase enzyme (THE) compared to black tea without the addition of the β-glucosidase enzyme (THK). This finding proves that the addition of the β-glucosidase enzyme in the early stages of fermentation can increase the aroma of black tea. Linalool and geraniol compounds significantly contribute to the aroma of tea products because linalool and geraniol, apart from having a pleasant odor, have a low threshold of 6 ppb and 7 ppb in water [7].

Besides volatile alcohol compounds, the breakdown of glycosidic bonds also produces nonalcoholic volatile compounds. β-Damascenone is one of the nonalcoholic compounds identified in the black tea sample in this study. This compound is hydrolyzed from 3-hydroxy-β-damascone. This present study reports that β-damascenone had a slightly lower concentration in the sample with the addition of the β-glucosidase enzyme (THE) than the sample without the addition of the β-glucosidase enzyme (THK). As discussed by Kinoshita et al., the release of β-damascenone was influenced by pH. The lower the pH, the more optimal the hydrolysis will be [21]. From this statement, the addition of the β-glucosidase enzyme can increase the pH of the fermented leaves so that the hydrolysis process is slightly inhibited.

Another volatile compound derived from the hydrolysis of glycosides is benzaldehyde. Although benzaldehyde has the pleasant odors of sweet, almond, and cherry notes, benzaldehyde does not contribute too much to the aroma of tea because of its high threshold of 750.9 ppb [22]. However, benzaldehyde is the main benzenoid in tea. There are two possibilities for benzaldehyde formation. The first is through the oxidation of benzyl alcohol [18], and the second is the hydrolysis of prunasin glycosides [12, 23]. The addition of the β-glucosidase enzyme can increase the hydrolysis process of prunasin so that it releases free benzaldehyde compounds. This is evidenced by the higher concentration of benzaldehyde in samples with the addition of the β-glucosidase enzyme (THE) than in samples without the addition of the β-glucosidase enzyme (THK).

3.3. The Volatile Compounds Identified in Black Tea. Apart from the hydrolysis of glycosides, the addition of the β-glucosidase enzyme also increases the concentration of volatile compounds from other precursors. Overall, the volatile compounds detected in black tea can be seen in Table 2.

A total of 131 volatile compounds were identified in black tea consisting of 17 alcohol compounds, ten ketones, seven ionone derivatives, eight esters, 16 aldehydes, 54 hydrocarbons, six phenolic compounds, two furanoids, four nitrogen compounds, two acid compounds, one sulfur compound, and four other compounds. Overall, the volatile compounds formed are products of enzymatic degradation of fatty acids, carotenoids, hydrolysis of glycosidic bonds, and Maillard reaction results.

Several compounds also increased from the volatile compounds detected in black tea due to the addition of β-glucosidase enzyme treatment. In black tea, with the addition of the β-glucosidase enzyme, 2-methylbutanol with a roasted wine and fruity odor increased significantly. The concentration of methyl salicylate was in black tea samples with β-glucosidase enzyme treatment up to 35.19 ppb, while in black tea without β-glucosidase enzyme treatment, it was only 5.6 ppb. Based on the research of Zhu et al., methyl salicylate has a threshold of 40 ppb [25]. The addition of the β-glucosidase enzyme in this study resulted in a methyl salicylate concentration that was almost close to the threshold of the compound. This further strengthens the fact that premium black tea can be produced by adding the β-glucosidase enzyme to black tea processing.

Another nonalcoholic compound identified in this study is methyl salicylate. Methyl salicylate is produced by hydrolysis of the glycoside compound Meβ-D-xylopyranosyl-(1-6)-β-D-glucopyranoside (gaultherin) [24]. Su et al. [9] explained that the addition of the β-glucosidase enzyme could increase the concentration of methyl salicylate by 2%. But in this study, the concentration of methyl salicylate increased significantly in black tea samples with β-glucosidase enzyme treatment up to 35.19 ppb, while in black tea without β-glucosidase enzyme treatment, it was only 5.6 ppb. Based on the research of Zhu et al., methyl salicylate has a threshold of 40 ppb [25]. The addition of the β-glucosidase enzyme in this study resulted in a methyl salicylate concentration that was almost close to the threshold of the compound. This further strengthens the fact that premium black tea can be produced by adding the β-glucosidase enzyme to black tea processing.

### Table 1: Effect of the β-glucosidase enzyme on glycosidic bound volatile compounds.

| No. | Compound          | CAS number | LRI  | THK (ppb) | THE (ppb) | Odor description* |
|-----|-------------------|------------|------|-----------|-----------|-------------------|
| 1   | 3-Hexenol         | 544-12-7   | 5.31 | 11.60     |           | Green leafy       |
| 2   | Benzyl alcohol    | 100-51-6   | 7.82 | 1.94      |           | Floral rose       |
| 3   | Phenylethyl alcohol | 60-12-8   | 12.97| 18.40     |           |                   |
| 4   | Linalool oxide    | 5989-33-3  | 6.73 | 15.32     |           | Earthy, floral sweet, woody |
| 5   | trans-Linalool oxide | 34995-77-2 | 8.31 | 41.60     |           | Floral            |
| 6   | Linalool          | 78-70-6    | 4.09 | 13.60     |           | Citrus floral sweet, woody |
| 7   | cis-Linalool oxide | 5989-33-3  | 0.82 | 2.21      |           | Earthy, floral sweet, woody |
| 8   | Epoxylinalool     | 14049-11-7 | 4.62 | 11.98     |           | Floral honey      |
| 9   | cis-Geraniol      | 106-25-2   | nd   | 1.49      |           | Sweet, neroli citrus magnolia |
| 10  | Geraniol          | 106-24-1   | 2.49 | 4.52      |           | Sweet floral, fruity, citrus |
| 11  | β-Damascenone     | 23726-93-4 | 0.86 | 0.80      |           | Apple, rose, honey, sweet |
| 12  | Benzaldehyde      | 100-52-7   | 4.08 | 6.61      |           | Sweet, almond, cherry |
| 13  | Methyl salicylate | 119-36-8   | 5.60 | 35.19     |           | Wintergreen, mint |

Note: THK: black tea from PGL 15 controls; THE: black tea treated with β-glucosidase. Ethyl decanoate was used as an internal standard. * References based on http://www.thegoodscentscompany.com/. nd: not detected.
Table 2: The volatile compounds identified in black tea.

| No. | Compound                        | CAS number  | LRI  | THK (ppb) | THE (ppb) | Odor description*          |
|-----|---------------------------------|-------------|------|-----------|-----------|-----------------------------|
|     | **Alcohols**                    |             |      |           |           |                             |
| 1   | 2-Methylbutanol                 | 137-32-6    | 6.97 | 21.29     |           | Roasted wine, fruity        |
| 2   | 3-Hexenol                       | 544-12-7    | 5.31 | 11.60     |           | Green leafy                 |
| 3   | 1-Octen-3-ol                    | 3391-86-4   | 983  | nd        | 1.35      | Mushroom, earthy green      |
| 4   | 2-Ethylhexanol                  | 104-76-7    | 1035 | 2.46      | 5.38      | Citrus fresh, floral sweet  |
| 5   | 2-Butyloctanol                  | 735273      | 1320 | nd        | 3.05      |                            |
| 6   | 7-Methoxy-1-naphthol            | 67247-13-6  | 1377 | 0.49      | nd        |                            |
|     | **Aromatic alcohol**            |             |      |           |           |                             |
| 7   | Benzyl alcohol                  | 100-51-6    | 1042 | 7.82      | 1.94      | Floral rose                 |
| 8   | Phenylethyl alcohol             | 60-12-8     | 1119 | 12.97     | 18.40     | Floral rose, dried rose flower |
| 9   | 2-tert-Butylcyclohexanol        | 13491-79-7  | 1294 | 1.34      | 1.55      | Pine camphor minty, patchouli |
|     | **Terpenoid alcohol**           |             |      |           |           |                             |
| 10  | Linalool oxide                  | 5989-33-3   | 1075 | 6.73      | 15.32     | Earthy, floral sweet, woody |
| 11  | trans-Linalool oxide            | 34995-77-2  | 1091 | 8.31      | 41.60     | Floral                      |
| 12  | Linalool                        | 78-70-6     | 1104 | 4.09      | 13.60     | Citrus floral sweet, blueberry |
| 13  | cis-Linalool oxide              | 5989-33-3   | 1174 | 0.82      | 2.21      | Earthy, floral sweet, woody |
| 14  | Epoxylinalool                   | 14049-11-7  | 1179 | 4.62      | 11.98     | Floral honey                |
| 15  | cis-Geraniol                    | 106-25-2    | 1232 | nd        | 1.49      | Sweet natural, neroli citrus |
| 16  | Geraniol                        | 106-24-1    | 1260 | 2.49      | 4.52      | Sweet floral, fruity, rose, citrus |
| 17  | Nerolidol                       | 7212-44-4   | 1568 | 3.50      | 6.52      | Floral green, citrus, woody |
|     | **Ketones**                     |             |      |           |           |                             |
| 18  | 2,3-Octanedione                  | 585-25-1    | 988  | 0.43      | 6.83      | Dill, asparagus, cilantro, herbal |
| 19  | 2,5,5-Trimethylcyclohex-2-enone | 42747-41-1  | 1301 | 2.42      | 2.91      |                             |
| 20  | 2-Benzylcyclohexanone           | 946-33-8    | 1468 | 0.10      | 0.69      |                             |
|     | **Aromatic ketone**             |             |      |           |           |                             |
| 21  | Acetophenone                     | 98-86-2     | 1062 | 1.76      | 0.78      | Sweet pungent, almond, acacia |
| 22  | Camphenone, 6-                  | 55659-42-2  | 1205 | 1.33      | 3.07      |                             |
| 23  | Geranyl acetone                 | 3796-70-1   | 1455 | 0.62      | 1.32      | Fresh green, fruity, rose, tropical |
| 24  | 2,4,5-Trimethylacetophenone      | 2040-07-5   | 1486 | 1.79      | 4.52      |                             |
| 25  | Benzophenone                    | 119-61-9    | 1634 | 1.12      | 1.05      | Rose, powdery, geranium     |
| 26  | 4′-Benzyloxycetophenone          | 782-92-3    | 1693 | 0.27      | nd        |                             |
| 27  | Hexahydrofarnesyl acetone       | 502-69-2    | 1847 | nd        | 0.26      | Jasmine, celery, woody      |
|     | **Ionone derivatives**          |             |      |           |           |                             |
| 28  | β-Damascenone                   | 23726-93-4  | 1386 | 0.86      | 0.80      | Apple, rose, honey, tobacco, sweet |
| 29  | α-Ionone                        | 127-41-3    | 1432 | 0.50      | 0.87      | Sweet, woody, floral, tropical fruity |
| 30  | Dihydro-β-ionone                | 17283-81-7  | 1444 | nd        | 0.15      | Earthy, woody, dry amber    |
| 31  | 1-(4-tert-Butylphenyl)propan-2-one | 81561-77-5  | 1453 | 0.42      | nd        |                             |
| 32  | 2,6-Di-tert-butyquinone         | 719-22-2    | 1471 | 1.59      | 2.56      |                             |
| 33  | α-Isomethyl ionone              | 127-51-5    | 1481 | 0.70      | 0.40      | Sweet orris, powdery, floral, woody |
| 34  | trans-β-Ionone                  | 79-77-6     | 1490 | 4.02      | 7.04      | Dry powdery, floral, woody, orris |
|     | **Esters**                      |             |      |           |           |                             |
| 35  | Methyl caprylate                | 111-11-5    | 1126 | nd        | 3.38      | Green, sweet orange         |
| 36  | cis-3-Hexenyl-α-methylbutyrate   | 53398-85-9  | 1238 | nd        | 0.62      | Fresh, green apple, sweet, pear |
| 37  | Isobornyl acetate               | 125-12-2    | 1289 | 1.43      | 0.87      | Herbal, woody, sweet        |
| 38  | 3-Hexenyl hexanoate             | 84434-19-5  | 1383 | 1.28      | nd        |                             |
| 39  | Ethyl decanoate                 | 110-38-3    | 1397 | 2.50      | 2.50      | Sweet, fruity, apple, grape  |
| 40  | Methyl laurate                  | 111-82-0    | 1529 | nd        | 1.96      | Creamy, coconut, mushroom   |
| No. | Compound                          | CAS number | LRI   | THK (ppb) | THE (ppb) | Odor description*                  |
|-----|-----------------------------------|------------|-------|-----------|-----------|------------------------------------|
| 41  | cis-3-Hexenyl benzoate            | 25152-85-6 | 1576  | 0.42      | 1.06      | Fresh green, leafy, floral, orchid |
| 42  | Methyl palmitate                  | 112-39-0   | 1928  | 0.24      | 1.01      | Waxy, fatty, orris                 |

**Aldehydes**

| No. | Compound                          | CAS number | LRI   | THK (ppb) | THE (ppb) | Odor description*                  |
|-----|-----------------------------------|------------|-------|-----------|-----------|------------------------------------|
| 43  | 2-Methylpropanal                  | 78-84-2    | 22.51 | 31.52     |           | Floral green                       |
| 44  | 2-Methylbutanal                   | 96-17-3    | 17.70 | 44.94     |           | Musty, cocoa, coffee, nutty        |
| 45  | Hexanal                           | 66-25-1    | 3.54  | 4.85      |           | Fresh green, grass leafy, sweaty   |
| 46  | 2-Hexenal, (E)-                   | 6728-26-3  | 2.15  | 4.63      |           | Green banana, fatty, cheesy        |
| 47  | Heptanal                          | 111-71-7   | 902   | 5.75      | 4.19      | Fresh green, herbal, wine-lee ozone|
| 48  | 2,4-Heptadienal, (E,E)-          | 4313-03-05 | 1012  | nd        | 1.25      | Fatty green oily, cake cinnamon    |
| 49  | Nonanal                           | 124-19-6   | 1106  | 20.61     | 39.88     | Rose, fresh orris, orange peel     |
| 50  | Decanal                           | 112-31-2   | 1207  | 0.82      | 0.74      | Sweet, orange peel, citrus floral  |

**Aromatic aldehyde**

| No. | Compound                          | CAS number | LRI   | THK (ppb) | THE (ppb) | Odor description*                  |
|-----|-----------------------------------|------------|-------|-----------|-----------|------------------------------------|
| 51  | Benzaldehyde                      | 100-52-7   | 962   | 4.08      | 6.61      | Sweet, almond, cherry              |
| 52  | Benzenecetaldehyde                | 122-78-1   | 1046  | 16.00     | 37.18     | Floral, sweet, clover, honey, cocoa|
| 53  | 2-Butenal, 2-phenyl               | 4411-89-6  | 1276  | 0.38      | 0.50      | Beany, honey cocoa, nutty radish   |
| 54  | 4-Indanecarbaldehyde              | 51932-70-8 | 1306  | 1.09      | 1.68      |                                   |
| 55  | 2-Hexylcinnamaldehyde             | 101-86-0   | 1753  | 0.33      | nd        | Fresh, floral green, jasmine, herbal|

**Terpenoid aldehyde**

| No. | Compound                          | CAS number | LRI   | THK (ppb) | THE (ppb) | Odor description*                  |
|-----|-----------------------------------|------------|-------|-----------|-----------|------------------------------------|
| 56  | β-Cyclocitral                      | 432-25-7   | 1223  | 3.22      | 5.22      | Sweet, fruity, tropical, saffron, rose|
| 57  | (R)-(+)-Citronellal                | 2385-77-5  | 1242  | nd        | 0.31      | Fresh herbal, citrus               |
| 58  | Citral                            | 5392-40-5  | 1274  | 0.39      | 0.29      | Sharp lemon sweet                  |

**Hydrocarbons**

| No. | Compound                          | CAS number | LRI   | THK (ppb) | THE (ppb) | Odor description*                  |
|-----|-----------------------------------|------------|-------|-----------|-----------|------------------------------------|
| 59  | 4-Methyldecano                     | 2847-72-5  | 1023  | 1.44      | 1.19      |                                   |
| 60  | 3,6-Dimethyldecano                 | 17312-53-7 | 1058  | 1.93      | 2.05      |                                   |
| 61  | 4-Methylundecane                   | 2980-69-0  | 1059  | 2.39      | 1.65      |                                   |
| 62  | 3,8-Dimethyldecano                 | 17312-55-9 | 1150  | 1.05      | 0.87      |                                   |
| 63  | 3-Methylundecane                   | 1002-43-3  | 1171  | 1.99      | 1.85      |                                   |
| 64  | Cyclododecanec                     | 294-62-2   | 1192  | 1.44      | 1.27      |                                   |
| 65  | Dodecano                          | 112-40-3   | 1201  | 30.48     | 31.71     |                                   |
| 66  | 2-Undecene, 5-methyl-              | 56851-34-4 | 1215  | 1.98      | 1.42      |                                   |
| 67  | 2-Methylundecanocyclopropane       | 1560-97-0  | 1264  | 4.26      | 2.35      |                                   |
| 68  | 5-Ethyl-2-methyloctano             | 62016-18-6 | 1280  | 4.39      | 2.50      |                                   |
| 69  | 3,5-Dimethylundecane               | 107770-99-0| 1340  | 0.69      | 0.63      |                                   |
| 70  | 2,3,7-Trimethyldecano              | 62238-13-5 | 1343  | nd        | 0.24      |                                   |
| 71  | 4-Ethyldecano                      | 1636-44-8  | 1347  | 1.08      | 1.40      |                                   |
| 72  | 3-Dodecanec, (Z)-                 | 7239-23-8  | 1365  | nd        | 0.75      |                                   |
| 73  | 3-Methyltridecan                   | 6418-41-3  | 1371  | 2.48      | 2.39      |                                   |
| 74  | Cyclopentane, nonyl-              | 2882-98-6  | 1386  | 0.87      | nd        |                                   |
| 75  | 1-Tetradecene                     | 1120-36-1  | 1392  | 1.82      | 1.66      |                                   |
| 76  | Tetradecanec                      | 629-59-4   | 1401  | 15.52     | 13.89     | Mild waxy                          |
| 77  | Cycloptadecanec                   | 295-17-0   | 1449  | 0.52      | 0.62      |                                   |
| 78  | 4,8-Dimethyltridecan              | 55030-62-1 | 1463  | 1.47      | 1.03      |                                   |
| 79  | Pentacontane, nonyl-              | 629-62-9   | 1500  | 0.71      | 26.58     | Waxy                               |
| 80  | Hexadecane                        | 544-76-3   | 1541  | 0.69      | 0.87      |                                   |
| 81  | Heptadecane                       | 629-78-7   | 1600  | 2.33      | 3.38      |                                   |

**Aromatic hydrocarbon**

| No. | Compound | CAS number | LRI   | THK (ppb) | THE (ppb) | Odor description*                  |
|-----|----------|------------|-------|-----------|-----------|------------------------------------|
| 82  | Naphthalene | 91-20-3  | 1186  | 12.06     | 12.22     | Pungent, dry tarry                 |
| No. | Compound         | CAS number | LRI   | THK (ppb) | THE (ppb) | Odor description*          |
|-----|------------------|------------|-------|-----------|-----------|-----------------------------|
| 83  | Eicosane         | 112-95-8   | 1710  | 0.24      | 0.44      | Waxy                        |
| 84  | Cadinene         | 5951-61-1  | 1336  | 1.88      | 1.67      |                             |
| 85  | Ionene           | 475-03-6   | 1360  | nd        | 0.23      |                             |
| 86  | 2,6-Dimethylnaphthalene | 581-42-0 | 1426  | nd        | 0.54      | Grass                       |
| 87  | 1,3-Diisopropynaphthalene | 1000374-05-2 | 1682  | 0.48      | nd        |                             |
| 88  | Cadalene         | 483-78-3   | 1686  | 0.76      | 1.21      |                             |
| 89  | 1,4-Diisopropynaphthalene | 1000374-05-7 | 1727  | 0.29      | 0.82      |                             |
| 90  | 2,6-Diisopropynaphthalene | 24157-81-1 | 1730  | 3.60      | 2.26      |                             |
| 91  | Copaene          | 3856-25-5  | 1379  | 1.16      | 1.06      | Woody spicy honey           |
| 92  | Modephene        | 68269-87-4 | 1383  | nd        | 0.77      |                             |
| 93  | Cyclosativene    | 22469-52-9 | 1374  | nd        | 0.84      |                             |
| 94  | Berkheyaradulene | 1000373-94-1 | 1391  | nd        | 1.09      |                             |
| 95  | Caryophyllene    | 87-44-5    | 1424  | 2.34      | 2.87      | Sweet, woody spice clove dry|
| 96  | Alloaromadendrene| 25246-27-9 | 1505  | 0.58      | 0.76      | Woody                       |
| 97  | Butylhydroxytoluene | 128-37-0   | 1518  | 2.69      | 4.60      | Mild camphor                |

**Terpenoid hydrocarbon**

| No. | Compound           | CAS number | LRI   | THK (ppb) | THE (ppb) | Odor description*          |
|-----|--------------------|------------|-------|-----------|-----------|-----------------------------|
| 98  | o-Xylene           | 95-47-6    | 7.75  | 9.18      |           | Geranium                    |
| 99  | D-Limonene         | 5989-27-5  | 1028  | 0.26      | 0.57      | Citrus orange, fresh sweet  |
| 100 | α-Cubebeene        | 17699-14-8 | 1354  | 0.90      | 0.77      | Herbal waxy                 |
| 101 | α-Longifolene      | 475-20-7   | 1409  | 1.25      | 1.31      | Sweet woody, rose, medical, fir |
| 102 | ε-Muurolene        | 30021-46-6 | 1415  | 1.32      | 2.41      |                             |
| 103 | β-Cubebeene        | 13744-15-5 | 1436  | nd        | 0.26      | Citrus, fruity, radish      |
| 104 | trans-α-Bergamotene | 13474-59-4 | 1438  | nd        | 0.14      | Woody, warm tea             |
| 105 | β-Guaiene          | 88-84-6    | 1442  | nd        | 0.27      | Sweet wooden, spicy, powdery|
| 106 | β-Bisabolene       | 495-61-4   | 1459  | 0.61      | 0.80      | Balsamic, woody             |
| 107 | γ-Gurjunene        | 22567-17-5 | 1478  | nd        | 0.95      | Musty                       |
| 108 | α-Farnesene        | 502-61-4   | 1511  | 1.45      | 3.07      | Citrus, lavender, bergamot, green |
| 109 | γ-Cadinene         | 39029-41-9 | 1521  | nd        | 3.26      | Herbal, woody              |
| 110 | α-Cedrene          | 469-61-4   | 1654  | nd        | 0.36      | Woody, cedar, sweet fresh   |
| 111 | α-Longipinene      | 5989-0805  | 1658  | nd        | 0.35      |                             |
| 112 | Dehydro-ar-ionene  | 30364-38-6 | 1358  | 0.54      | 0.32      | Licorice                    |

**Phenolic compounds**

| No. | Compound          | CAS number | LRI   | THK (ppb) | THE (ppb) | Odor description*          |
|-----|-------------------|------------|-------|-----------|-----------|-----------------------------|
| 113 | o-Cresol          | 95-48-7    | 1165  | 1.85      | 1.15      | Musty, plastic, medicinal, leathery |
| 114 | Eugenol           | 97-53-0    | 1364  | 1.44      | 0.31      | Sweet spicy, clove woody    |
| 115 | Methyl salicylate | 119-36-8   | 1199  | 5.60      | 35.19     | Wintergreen, mint           |
| 116 | 2,4-Di-tert-butylphenol | 96-76-4 | 1524  | 2.05      | 2.52      |                             |
| 117 | 4-(1-Hydroxyallyl)-2-methoxyphenol | 112465-50-6 | 1531  | 2.28      | 0.51      |                             |
| 118 | 2,4,6-Tri-tert-butylphenol | 732-26-3 | 1910  | nd        | nd        |                             |

**Furanoid**

| No. | Compound          | CAS number | LRI   | THK (ppb) | THE (ppb) | Odor description*          |
|-----|-------------------|------------|-------|-----------|-----------|-----------------------------|
| 119 | Furan, 2-pentyl-  | 3777-69-3  | 992   | 2.00      | 5.06      | Fruity, green               |
| 120 | Furan, 3-phenyl-  | 13679-41-9 | 1226  | nd        | 1.73      | Floral                      |

**Nitrogenous compounds**

| No. | Compound          | CAS number | LRI   | THK (ppb) | THE (ppb) | Odor description*          |
|-----|-------------------|------------|-------|-----------|-----------|-----------------------------|
| 121 | 5-Methylthiazole  | 3581-89-3  | 1246  | 1.55      | 2.44      |                             |
| 122 | Dihydroactinidilide | 17092-92-1 | 1538  | 0.62      | nd        |                             |
| 123 | 2-Methylphenothiazine | 5828-51-3 | 1648  | nd        | 0.92      |                             |
| 124 | Caffeine          | 58-08-2    | 1858  | 1.76      | 3.35      | Odorless                    |
odor, was also increased in black tea with the enzyme β-glucosidase. The alcohol group compound, which also increases due to the addition of the β-glucosidase enzyme, is nerolidol. Nerolidol is a volatile compound from carotenoid precursors with a floral green and citrus odor. In oolong tea, nerolidol is formed due to the photooxidation of phytoflavone [26]. But in this study, the nerolidol compound also increased in black tea with the addition of β-glucosidase enzyme treatment.

Many aldehyde compounds are found in the black tea aroma and have a significant contribution. The primary formation of black tea aroma is from glycoside hydrolysis and lipid degradation [2]. Aldehyde compounds from lipid precursors that increase with the treatment of the β-glucosidase enzyme include 2-methylpropanal with a green floral odor, 2-methylbutanal with a musty, cocoa, coffee, and nutty odor, and nonanal with a rose, fresh orris, and orange peel odor. Not all volatile compounds from lipid precursors have pleasant scents. Some of them have fatty aromas such as hexanal (fresh green, grassy), E-2-hexenal (green, banana, fatty, and cheesy), and (E,E)-2,4-heptadienal (fatty, green, and oily). The aldehyde class of compounds is also divided into aromatic aldehydes and terpenoid aldehydes. Aromatic aldehyde compounds such as benzeneacetaldehyde with a floral, sweet, clover, honey, and cocoa odor in black tea with β-glucosidase enzyme (THE) treatment had the highest concentration in the three black tea samples, namely, 37.18 ppb. Likewise, terpenoid aldehyde compounds include β-cyclocitrinal (sweet, fruity, tropical, saffron, and rose odor) with a concentration of 5.22 ppb. Zhang et al.’s study also showed an increase in the concentration of aromatic aldehyde compounds due to the β-glucosidase enzyme treatment [27].

Terpenoid hydrocarbon compounds increased with the addition of the β-glucosidase enzyme such as o-xylene with a geranium odor, D-limonene with a citrus, fresh, and sweet aroma, α-farnesene with a citrus, lavender, bergamot, and green scent, and γ-cadinene with a woody and herbal odor. These compounds had a higher concentration in the THE sample than in the THK sample. α-Farnesene and geranyl acetone from the ketone group are formed due to the photooxidation of phytoflavone [26].

The furanoid compounds detected in black tea are furan, 2-pentyl- and furan, 3-phenyl-. Furan compound 2-pentyl- with a fruity odor has the highest concentration in the THE sample, namely, 5.06 ppb. Meanwhile, furan, 3-phenyl- with a floral odor was not detected in THK samples but detected in THE samples with a concentration of 1.73 ppb. However, it cannot be concluded that the increase in the concentration of furan, 3-phenyl- is the result of the addition of the β-glucosidase enzyme. As discussed by Zheng et al. and Qi et al., some furans can be formed due to the Maillard reaction, namely, the interaction between amino acids and reducing sugars [1, 28].

### 4. Conclusion

One hundred thirty-one volatile compounds were identified in black tea, and 13 are from glycoside precursors that increased due to the addition of the β-glucosidase enzyme. They were 3-hexenol, benzyl alcohol, phenylethyl alcohol, linalool oxide, trans-linalool oxide, linalool, cis-linalool oxide, epoxylinalool, cis-geraniol, geraniol, β-damascenone, benzaldehyde, and methyl salicylate.

### Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

### Disclosure

This research was a part of the research entitled “Selection and suitability of several Pagilaran superior tea clones as a premium tea raw material through metabolomics components analysis.”

### Conflicts of Interest

The authors declare no conflict of interest.

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**Table 2: Continued.**

| No. | Compound | CAS number | LRI | THK (ppb) | THE (ppb) | Odor description* |
|-----|----------|------------|-----|-----------|-----------|--------------------|
| **Acids** | | | | | | |
| 125 | 2-Ethylhexanoic acid | 149-57-5 | 1139 | 4.47 | 1.46 | Unpleasant, rancid, sour fatty |
| 126 | n-Decanoic acid | 334-48-5 | 1283 | 0.56 | 0.44 | |
| **Sulfur compound** | | | | | | |
| 127 | 2-Ethyl-5,7-dimethyl-1-benzothiophene | 18428-05-2 | 1422 | 0.57 | nd | |
| **Others** | | | | | | |
| 128 | 1-Oxaspiro[4.5]dec-6-ene,2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene | 36431-72-8 | 1315 | nd | 0.76 | Tea, herbal, green, wet tobacco leaf, woody, spicy |
| 129 | Diphenyl ether | 101-84-8 | 1405 | 0.90 | 0.78 | Geranium leaf, green |
| 130 | Cyclopropane, nonyl- | 74663-85-7 | 1474 | 0.35 | 0.27 | |
| 131 | Biphenyl-1, 2′, 5′, 5′-tetramethyl- | 3075-84-1 | 1716 | 0.34 | 0.58 | |

Note: THK: black tea from PGL 15 controls; THE: black tea treated with β-glucosidase; THP: black tea from the Pagilaran factory. Ethyl decanoate was used as an internal standard. *References based on http://www.thegoodscentsofcompany.com/. nd: not detected.
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