https://doi.org/10.48048/tis.2022.1526

Effect of Drying Time and Temperature to the Chemical Properties and Enzymatic Activities Related to the β-ocimene Production in *Syzygium polyanthum* Leaves

Bima Putra Pratama[1], Yudi Pranoto[1,*], Supriyadi[1] and Respati Tri Swasono[2]

[1]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Bulaksumur, Yogyakarta 55281, Indonesia
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

([*]Corresponding author's e-mail: pranoto@ugm.ac.id)

Received: 25 December 2021, Revised: 13 February 2022, Accepted: 25 February 2022, Published: 6 November 2022

Abstract

As a key volatile compound, the β-ocimene volatile compound in Salam leaves may change due to processing. Thus, this study aims to examine the effect of the drying process on the water activity, pH, total soluble protein values, 1-deoxy-D-xylulose-5-phosphate synthase, geranyl diphosphate synthase, and monoterpene synthase enzyme activities, and total β-ocimene due to variations in drying time (0, 1, 2, 3 and 4 days) and temperature (30, 40, 50 and 60 °C). The results showed that all parameters significantly decreased day by day, especially at higher temperatures. Meanwhile, for the temperatures of 40 and 50 °C, the enzyme activity parameters were increased until the 1st drying day. The drying process with a temperature of 40 °C gives the highest value of β-ocimene, reaching 139.62 µg/mL Salam leaves essential oil. These results were entirely satisfactory, and research conditions can be a reference for 4 days drying process of Salam leaves.

Keywords: Salam leaf, Drying process, Chemical properties, Enzyme activity, β-ocimene

Introduction

Salam leaf comes from the Salam plant (*Syzygium polyanthum*). This leaf is one of the various leaves often used in Indonesia as flavoring agents for foods. Salam leaves are easy to be found in Southeast Asia regions, such as Myanmar, Thailand, Malaysia and Indonesia because they live in tropical regions [1]. Its usage as a flavoring agent is due to the presence of essential oil with a large number of terpenoid compounds in the leaf extract. In the previous research, it is stated that there are several terpenoid compounds in the essential oil of Salam leaf, including β-ocimene, α-pinene, cis-4-decenal, octanal, farnesol, nerolidol and α-humulene [2]. As for research related to β-ocimene in Salam leaves, it is explained that this affects the level of preference of consumers, especially from the fresh aroma produced and even the taste when mixed into food [3]. The β-ocimene is a terpenoid compound that is also explained as a critical aroma compound of Salam leaves which is marked by its most minor limit of detection (LOD - is the minimum concentration for a compound that an organism sensorily can detect) value in Salam leaves [4]. Meanwhile, for its functional properties, several studies have stated that β-ocimene has the ability as an antitumor, anticonvulsant, antifungal and as a pesticide [5-8]. If applied in agriculture, the aroma produced by β-ocimene is a pheromone to attract bees in pollination [9].

The amount of terpenoid compounds in the essential oil of a plant is influenced by the metabolic processes that occur [10]. There are 2 pathways of terpenoid metabolism in plants, the mevalonic acid pathway (MVA pathway) and the deoxyxylulose phosphate pathway (DXP pathway) [10]. The MVA pathway specializes in the production of sesquiterpenoids (C15) and triterpenoids (C30), while the DXP pathway tends to produce monoterpenoids (C10), diterpenoids (C20) and carotenoids (C40) [11]. As a monoterpenoid compound, β-ocimene has been proven to be produced by the DXP pathway [12]. The concentration of β-ocimene is affected by the enzyme's activity that acts on this pathway, such as 1-deoxy-d-xylulose-5-phosphate synthase (DXS) enzyme, geranyl diphosphate synthase (GPPs) enzyme, and monoterpene synthase (MTPs) enzyme activities [13]. Thus, environmental conditions that affect the activity of these enzymes also affect the metabolism and concentration of β-ocimene produced.

Traditionally, Salam leaves are dried before they can be used. The drying process can affect the activities of the enzymes of the leaves. There is also a high possibility for the process to impact the properties of the bioactive compounds of the leaves. It was stated that in the drying process of pineapple (*Ananas comosus*), the temperature of 40 °C was able to keep the bromelain enzyme activity unchanged for a longer time when compared to higher temperatures [9]. Then, on dried *Carica papaya* fruit, the highest physical and chemical characteristics were obtained at a drying temperature of 40 °C [15]. Also, for *Persea Americana* leaves, a temperature of 50 °C could reduce the activity of the polyphenol oxidase (PPO) enzyme [16]. Thus, the drying time is a variable that also needs to be evaluated. From the reasons explained, this research was conducted with the aim of examining the effect of variations in temperature and time of drying Salam leaves on chemical parameters, enzymes activities related to β-ocimene metabolism, and β-ocimene yield as an evaluation of the drying process and find the best condition for maintaining the quality of Salam leaves, especially seen from the highest concentration of β-ocimene produced related with their usage as a natural flavoring agent.

Materials and methods

Materials

Fresh Salam leaves were used as the main material in this study were purchased from CV Bina Agro Mandiri, Bantul, D.I. Yogyakarta, and Indonesia. The purchased Salam leaves were selected from the old part of the leaves (shoots+8) that have higher bioactive components than younger ones [17]. The selected leaves were those which were not physically damaged. The size of the leaves used was equalized with a length of 9 - 12 cm and a width of 4 - 6 cm. Chemicals and tools for each stage are described in the following stages.

Treatments

Factors in this study were the variations in temperature and drying time of the Salam leaves. Fresh Salam leaves approximately 100 - 300 g were placed on an aluminum tray for the drying process. The variations for drying temperature were 30, 40, 50 and 60 °C. Meanwhile, the drying time was varied for 0, 1, 2, 3 and 4 days. Observations on temperature variations were performed using an 80×80×80 cm^3 box system equipped with 1. Several 5-watt incandescent lamps were used as a heat source; 2. A blower to maintain airflow; and 3. A thermometer to monitor the temperature in the box. Then, Salam leaves were taken from each variation combination of drying time and temperature to be analyzed for their water activity, total soluble protein, pH level, the activity of DXS, GPPs and MTPs enzymes, as well as their total β-ocimene metabolite products.

Water activity analysis

The sample's water activity value (aw) was measured with a water activity meter (with AquaLab CX-2 water activity meter). Before the instrument was used, it was calibrated first by measuring the aw value of several standard salts, including sodium chloride (aw value = 0.7509) and potassium sulfate (aw value = 0.97) at 30 °C. After that, 5 g of Salam leaves that have been treated were crushed with a mortar and put into the material plate of the aw meter. Then, it was measured within 15 min until the results of the aw value were shown and can be read on the digital display of the aw value on the aw meter [18].

Total soluble protein analysis

The total soluble protein of Salam leaf crude enzyme extract was analyzed using the Bradford test. A total of 10 microliters of crude enzyme extract was diluted with 490 L aquabides and mixed with 500 L Bradford's reagent. Then, the mixture was incubated for 5 min at room temperature. A blue-colored complex was produced from the mixture due to the formation of protein-dye complexes in an acidic environment. Then, the intensity of the produced blue color was measured with a UV-Vis spectrophotometer (Spectrophotometer Shimadzu UV 1800) at 595 nm. As a standard, bovine serum albumin solutions with concentrations varying from 1 to 15 g/mL (5 concentration series) were calibrated with the same mixture to create a calibration curve [19].

PH analysis

The treated Salam leaves were extracted with 5 g mixed with 25 mL of distilled water. Then, the mixture was blended for 1 min, which was repeated 3 times to get a smooth blender result. Then, the pH testing was proceeded by inserting the pH meter probe into the mixture. After that, the pH value of the

leaf extract can be seen in the pH indicator. For more accurate results, the pH meter was calibrated before it was used by using a buffer with a known pH value [20].

Crude enzyme extract preparation

A total of 10 g of fresh Salam leaves were mixed with 20 mL of cold acetone at 4 °C. Then, the mixture was blended (with Philips Blender 5000 series) 4 times for each sample with a 30-second interval per repetition. Then, the juice without the blended pulp was left for 5 min, placed in a falcon tube, then centrifuged at 3000 g for 30 min (with LMC-3000 Laboratory Centrifuge). After that, the produced supernatant was mixed with 0.35 g potassium dihydrogen phosphate and 0.02 M glutathione powder, vortexed, and was left for 1 h at 4 °C. Then, it was centrifuged again until the supernatant became crude enzyme extract for it to be analyzed [21].

DXS, GPPs and MTPs enzyme activities analysis

The main material of this analysis was the crude leaf enzyme extract as described above. For DXS enzyme activity, the analysis process was performed using the method developed on the reference [22]. Then, the activity of the GPPs enzyme was analyzed using the method in a study conducted before [23]. After that, the analysis of activity of MTPs enzyme was proceeded based on the method from a previous study [24].

Total β-ocimene quantification

Salam leaves, after being treated, were distilled by using the water distillation method. A total of 10 g of Salam leaves were mixed with 50 mL of distilled water and placed into the distilled water system. The distillation process lasted for 3 h. The distilled essential oil was quantified for its total β-ocimene using a standard β-ocimene solution with various concentrations of 10 - 50 g/mL (5 variations in concentration). Each sample was injected with a total of 1 µL into Gas Chromatography-Mass Spectrometry (GC-MS - Shimadzu GC17A - MS QP-5000) equipped with a CP Sil 5 CB column. The settings of the GC-MS instrument consisted of the initial column temperature at 60 °C and the final temperature at 250 °C, the temperature increasing rate to 10 °C/min, with helium gas as the carrier gas, ionizing electron impact type, and with ionization energy of 70 eV [24].

Statistical analysis

The method used was an experimental method with Completely Randomized Design (CRD). The data were processed using IBM SPSS Statistics 21.0. If the test results showed a different effect (Fcount > Ftable), the statistical analysis was continued using Duncan Multiple Range Test (DMRT) analysis with a significance level of 95 %.

Results and discussion

Results

The data in **Table 1** related to the results of the water activity value show that there are some changes in the value of water activity of leaves during the drying process. The table shows that the leaves' water activity (aw) value changed significantly from day to day, with the highest aw value on day 0, which decreased significantly until day 4 of the drying process. Meanwhile, for the drying temperature at 30 °C, the aw value decreased significantly until the 2nd day and then continuously decreased faintly until the 4th day of the drying process. Meanwhile, for higher temperatures, such as at temperatures of 40, 50 and 60 °C, the aw value decreased significantly from day 0 to day 4 of drying. As for the higher the temperature, the greater the difference between the aw values from day to day. The range of aw values in Salam leaves from all treatments ranged from 0.12 to 0.77. Based on the reference, the aw value that uses for enzymatic activity is between 0.25 - 0.75 [25], so that this water activity data is possible to be a factor that influences other chemical parameters in this study.

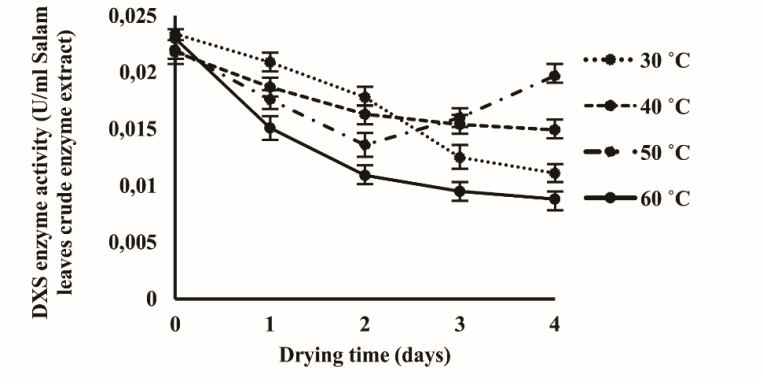
Table 1 Data on chemical parameters of leaf water activity, pH value, and total soluble protein of Salam leaf extract at different drying times and temperatures.

Chemical Parameters	Drying Temperature (°C)	Drying Time (days)				
		0	**1**	**2**	**3**	**4**
Water activity value	30	0.77 ± 0.03^{a}	0.72 ± 0.10^{b}	0.69 ± 0.06^{bc}	0.68 ± 0.01^{c}	0.66 ± 0.07^{c}
	40	0.75 ± 0.01^{a}	0.68 ± 0.15^{b}	0.62 ± 0.30^{c}	0.57 ± 0.03^{d}	0.55 ± 0.03^{d}
	50	0.75 ± 0.07^{a}	0.61 ± 0.02^{b}	0.53 ± 0.29^{c}	0.39 ± 0.01^{d}	0.33 ± 0.03^{e}
	60	0.76 ± 0.05^{a}	0.51 ± 0.05^{b}	0.39 ± 0.17^{c}	0.23 ± 0.05^{d}	0.12 ± 0.08^{e}
pH value	30	7.20 ± 0.28^{a}	7.00 ± 0.45^{a}	6.50 ± 0.22^{b}	5.76 ± 0.21^{c}	4.87 ± 0.20^{d}
	40	7.14 ± 0.37^{a}	6.69 ± 0.27^{a}	6.24 ± 0.14^{a}	5.71 ± 0.28^{b}	5.65 ± 0.20^{b}
	50	7.21 ± 0.15^{a}	6.40 ± 0.35^{b}	5.76 ± 0.40^{c}	6.44 ± 0.22^{d}	6.62 ± 0.38^{d}
	60	7.20 ± 0.24^{a}	6.65 ± 0.15^{b}	6.35 ± 0.46^{b}	6.56 ± 0.55^{b}	6.97 ± 0.11^{c}
Total soluble protein (mg/g crude enzyme extract)	30	32.72 ± 3.10^{a}	26.10 ± 5.32^{b}	20.10 ± 2.15^{c}	12.00 ± 2.74^{d}	10.11 ± 2.88^{e}
	40	31.22 ± 1.92^{a}	28.66 ± 2.87^{b}	24.10 ± 3.45^{c}	17.89 ± 2.76^{d}	20.11 ± 3.04^{d}
	50	32.76 ± 2.09^{a}	25.65 ± 2.86^{b}	22.10 ± 2.30^{c}	24.77 ± 1.55^{d}	25.44 ± 4.20^{d}
	60	33.65 ± 1.15^{a}	24.80 ± 1.20^{b}	19.70 ± 3.10^{c}	14.28 ± 2.50^{d}	7.89 ± 2.13^{e}

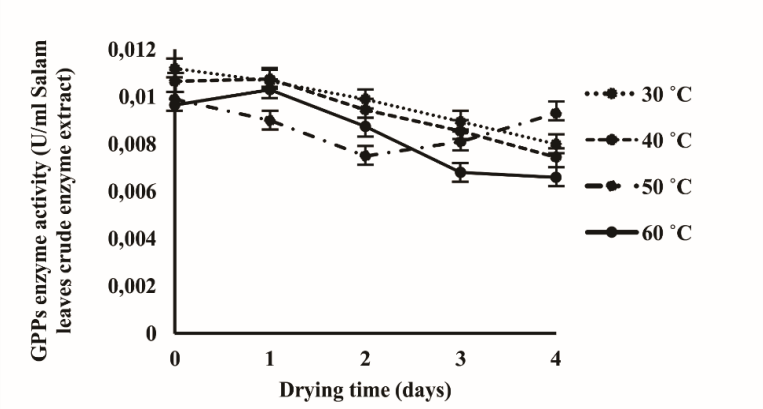
Superscript lowercase letters indicate significant differences between data in 1 row due to variations in drying time treatment with a significance level of 5 %.

Table 1 shows that the pH value of Salam leaf extract after being given drying treatment was decreased ranging from pH 7.21 (day 0) to pH 4.87 (day 4) of the drying period. Related to the treatment at different drying temperatures, the 30 and 40 °C continued to decrease in pH value along with the drying time. Meanwhile, for temperatures of 50 and 60 °C, the pH value of the leaves extract showed irregular patterns. For the total soluble protein, shown in **Table 1**, Salam leaf extract is containing 33.65 mg/g crude enzyme extract at its fresh condition and 7.89 mg/g as the lowest concentration of the soluble protein after the treatment. It can be seen that the total soluble protein value of Salam leaves decreased significantly until the last day of the drying process, especially at temperatures of 30 and 60 °C, the changes could be related to pH values and high temperatures that can affect the solubility level of leaf proteins which are explained further in the discussion section. As for the drying temperature of 40 and 50 °C from day 2 to day 4 of drying, the total soluble protein value tends to be maintained.

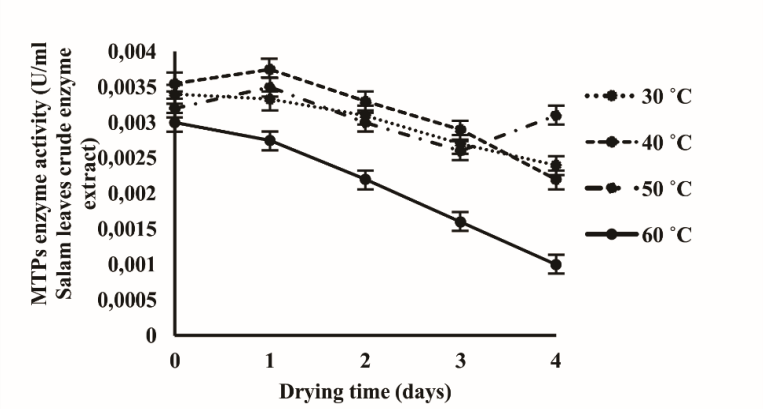
The DXS, GPPs and MTPs enzymes activities change following the drying period, as shown in **Figure 1**. The data in **Figure 1**(**A**) indicates that DXS Salam leaves' enzyme activities score ranged from 0.088 to 0.0235 U/mL of the Salam leaves crude enzyme extract. The graph also shows decreased enzyme activity during the drying process occurred in all variance temperatures. However, at 40 °C, the enzyme activity could be maintained on the 3rd and 4th drying day. Even at 50 °C, DXS enzyme activity increased at the end of drying, especially when compared to temperatures of 30 and 60 °C which continued to decrease at the variation of the drying time. Another data experiment shows the value of GPPs enzyme activity of Salam leaf was in the range of 0.0055 to 0.0112 U/mL Salam leaves crude enzyme extract (**Figure 1**(**B**)). At 30 and 50 °C, the enzyme activity of GPPs immediately decreased from day 0 to the 1st day of drying; for 40 and 60 °C drying temperature, the activity increased not significantly at the beginning of drying, and decreased drastically to the end of drying process; especially at a temperature of 50 °C, the activity increased significantly at the end of drying.



(A)



(B)



(C)

Figure 1 Graph of data from the analysis of enzyme activity and its changes in: (A) DXS enzyme, (B) GPPs enzyme and (C) MTPs enzyme Salam leaves crude enzyme extract on differences in drying time and temperature.

The MTPs enzyme activity is shown in **Figure 1(C)**. The values are changing in the range of 0.0010 to 0.0034 U/mL Salam leaves crude enzyme extract. Based on the graph, the enzyme activity of MTPs at temperatures of 40 and 50 °C has a higher graph when compared to temperatures of 30 and 60 °C. This indicated that the MTPs enzyme activity was higher at 40 and 50 °C. A temperature of 40 °C was able to produce a higher value of enzyme activity than 50 °C at the beginning of drying, while on the 4th day/last variation of drying, the enzyme activity of MTPs at 50 °C was the highest and even increased when compared to the drying temperature of other Salam leaves. Then, as initial information on the volatile compounds of Salam leaves that we used, we attach the results of the GC-MS analysis of its volatile

compound profiles. The chromatogram results are shown in **Figure 2** and described in **Table 2**. After the GC-MS results were obtained, both before and after drying, the β-ocimene quantification process was carried out using the β-ocimene solution, and the result is shown in **Figure 3**.

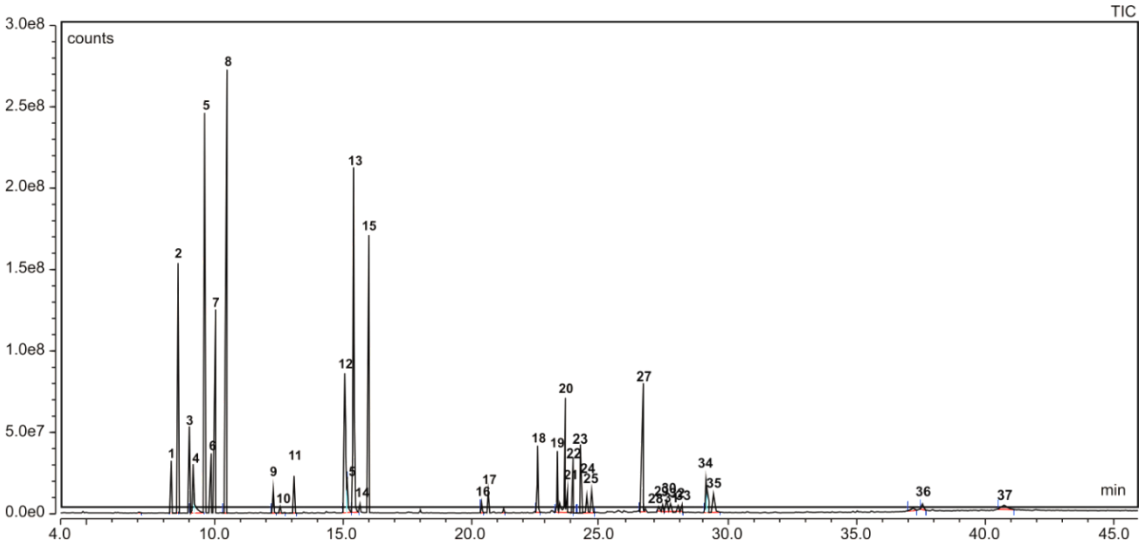


Figure 2 Chromatogram of volatile compounds of Salam leaf essential oil.

Table 2 Description of the volatile compound profile of Salam leaf essential oil.

No.	Ret.Time (min)	Volatile compounds	Rel. Area (%)	Groups
1	7,08	α-Pinene	1,02	monoterpene
2	7,11	Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl-	6,09	monoterpene
3	8,34	β-Pinene	2,02	monoterpene
4	8,79	β-Myrcene	1,03	monoterpene
5	9,24	Octanal	12,11	aldehyde
6	9,86	o-Cymene	1,04	monoterpene
7	9,96	D-Limonene	5,27	monoterpene
8	10,24	β-Ocimene	12,01	monoterpene
9	12,28	Linalool	2,33	oxygenated monoterpene
10	12,29	Nonanal	0,80	aldehyde
11	13.17	2,2-Dimethyl-3-(3-methylpenta-2,4-dienyl)oxirane	1,86	oxygenated monoterpene
12	15,15	cis-4-Decenal	3,06	aldehyde
13	15,23	4-Decenal, (E)-	10,02	aldehyde
14	15,29	α -Terpineol	0,89	oxygenated sesquiterpene
15	15,55	Decanal	7,88	aldehyde
16	20,42	.alfa.-Copaene	0,72	sesquiterpene
17	20,75	n-Decanoic acid	0,87	ester
18	22,58	Humulene	1,05	sesquiterpene
19	23,35	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	1,74	sesquiterpene
20	23,46	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4aa,7a,8aβ)]-	3,01	sesquiterpene
21	23,62	Guaia-1(10),11-diene	0,70	sesquiterpene
22	23,67	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2a,4aa,8aβ)]-	1,93	sesquiterpene
23	24,26	(-)-a-Panasinsen	2,10	sesquiterpene
24	24,27	a-Maaliene	1,11	sesquiterpene
25	24,36	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	1,20	sesquiterpene
26	26,38	Caryophyllene oxide	1,05	oxygenated sesquiterpene
27	26,65	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	3,92	sesquiterpene

No.	Ret.Time (min)	Volatile compounds	Rel. Area (%)	Groups
28	27,06	Isoaromadendrene epoxide	0,14	oxygenated sesquiterpene
29	27,08	Cubenol	0,34	oxygenated sesquiterpene
30	27,43	.tau.-Cadinol	0,62	oxygenated sesquiterpene
31	27,70	trans-Z-a-Bisabolene epoxide	0,32	oxygenated sesquiterpene
32	27,90	Globulol	0,23	oxygenated sesquiterpene
33	27,91	(-)-Globulol	0,13	oxygenated sesquiterpene
34	29,21	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	1,01	oxygenated sesquiterpene
35	29,42	trans-Farnesol	0,55	oxygenated sesquiterpene
36	37,51	Phytol	0,64	oxygenated monoterpene
37	40,73	Octadecan e, 3-ethyl-5-(2-ethylbutyl)-	0,06	ester

In the profile of Salam leaves volatile compounds, which shown in **Figure 2** and described in **Table 2**, Salam leaves essential oil were composed of 33.87 % aldehydes, 28.48 % monoterpenoid, 17.48 % sesquiterpenoid, 4.83 % oxygenated monoterpenoid, 5.28 % oxygenated sesquiterpenoid, and 0.93 % esters. Based on GC-MS results, then total β-ocimene was quantified using standard ocimene solution which resulted in a standard curve equation $y = 41.48x - 12.987$. The notation x is the concentration of the standard ocimene solution, and y is the relative percent of ocimene resulting from the GC-MS analysis. From these equations, the results of β-ocimene quantification are shown in **Figure 3**.

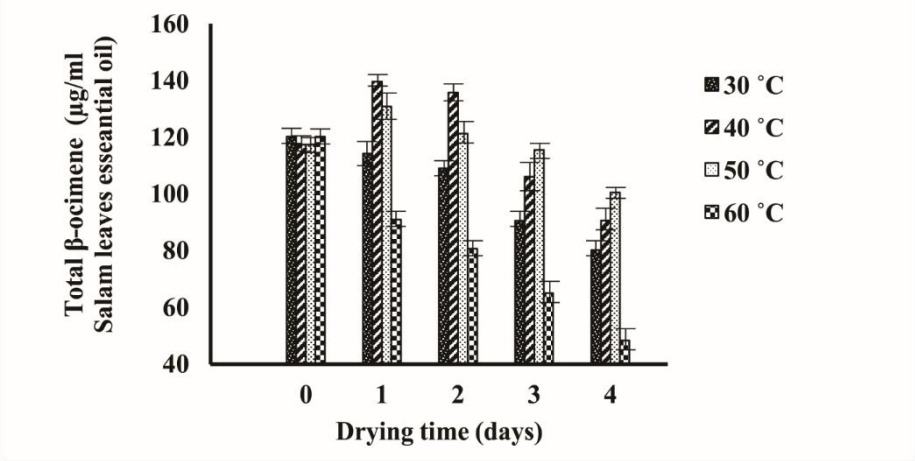


Figure 3 Graph of the results of quantification of total β-ocimene of Salam leaf essential oil based on differences in temperature and time of the drying process of Salam leaves.

In the last data, the graph in **Figure 3** shows that the maximum value of total β-ocimene in Salam leaves essential oil was found at a temperature of 40 °C on the 1st day of drying as much as 139.62 µg/g oil. The lowest value was found at a drying temperature of 60 °C for 4 days of the drying with a total value of 48.31 µg/g oil. The total value of β-ocimene decreased from day to day, and these results occurred mainly at temperatures of 30 and 60 °C. Meanwhile, at 40 and 50 °C, the value increased until the 1st drying day, then decreased not significantly on the 2nd day of drying, and decreased significantly until the 4th day of drying. The highest total β-ocimene value was obtained at a temperature of 40 °C, while temperature of 50 °C can give the total value of β-ocimene at a higher level on days 3 and 4 of drying than other temperature variations.

Discussion

In the results section, especially for the water activity values in **Table 1**, a temperature of 60 °C is more capable of giving a sharp decrease from day to day in the drying process, followed by temperatures of 50, 40 and 30 °C for the effect of decreasing the aw value from high to low respectively. This happens because theoretically, the higher temperature of drying, the lower the water content in the environment which causes the difference in the water content of the environment with the material to be even greater

[26]. As is known, the relationship between the water content of a natural material and the rate of enzymatic reactions that take place in it is known to be determined by the level of the aw value of the material. Water activity value described must be above 0.25 to still provide good conditions for the enzymatic reaction to take place. Meanwhile, for preservation purposes, an aw value below 0.25 can increase the shelf life of a material [27]. Meanwhile, in this study, it is known that up to a temperature of 50 °C, the effect on the aw value is still safe for the enzymatic process to take place. However, chemical factors also affect the activity of enzymes as described in the next paragraph.

During the drying process, there will also be an enzymatic process that produces products in the form of organic acids [28]. Hence, there is a decrease in pH in Salam leaves during the drying process shown in **Table 1** for the pH value section, especially at the beginning of the drying process. At 30 °C, the pH value continued to decrease until the 4th drying day. In contrast, for temperatures above 30 °C, the pH value gradually increased at the end of the drying process. The higher the temperature and the longer drying time causes the evaporation of several organic acids so that the acidity of the material decreases [29]. Then for the next test result is total soluble protein. The testing mechanism with the Bradford test is that the dye forms a complex with the protein carboxyl group with Van der Waals strength and the amino group through electrostatic interactions. Changes in protein conformity and binding in the protein will change the test results [30]. The presence of extreme aw and pH values can cause conformity changes in the enzyme protein. That is why at the start of the drying process, all yield data decreased significantly for protein solubility at all drying temperatures. At 30 °C, it continued to decrease because the pH value continued to decrease even though the aw conditions were still supportive for the last day of drying. At temperatures of 40 and 50 °C, the protein solubility of the enzyme increased because the aw and pH values were still supportive. Meanwhile, at a temperature of 60 °C, even though the pH value increased, the aw of the material was below 0.25, so it was possible that the protein had been denatured and the solubility was very low. In addition to causing changes in the solubility of leaf proteins, these changes can also affect the activity of the enzyme as a factor that affects the active site of the enzyme [31].

As for DXS enzyme activity, based on previous research, the optimal value is at pH value of 7.5 and temperature of 42 °C [32]. GPPs enzymes have an optimal pH of 6.5 and an optimal temperature of 40 °C for their enzyme activity [33]. The MTPs enzyme activity is optimal at a pH close to neutral and a temperature of around 45 °C [34]. The results in this study did not have a significant difference from those contained in the reference. The enzyme activity of DXS, GPPs and MTPs decreased slowly within 2 days of drying at a temperature of 30 °C and then decreased drastically to the 4th day of drying due to the changes of aw and pH values, which is related to changes in protein solubility as described in the previous paragraph. The decreased total protein solubility leads to a decrease in enzyme activity in the protein [31]. Meanwhile, for temperatures of 40 and 50 °C, the enzyme activity decreased at the beginning of the drying because the pH and aw value decreased significantly, then increased at the end of drying for 50 °C. It is linear with changes in the degree of protein solubility at 40 and 50 °C. At a temperature of 60 °C, enzyme activity decreased significantly since the 1st day of drying and continued to decline until the last drying day because the aw value could not support the presence of enzymes for activity.

Then on the results of the GC-MS analysis, the profile of volatile compounds shows a higher percentage of monoterpenoids in Salam leaf essential oil than the sesquiterpenoids, as follows in the literature. That fact is because salam leaves have β-ocimene, which is monoterpenoids as key volatile compounds, then also the DXP pathway could be more dominant in producing terpenoid compounds [35]. Then, as for the results of the total β-ocimene, they were consistent with the results on enzyme activity. At a temperature of 40 and 50 °C of drying, the results had a higher value when compared to other temperature and drying time variations. At a temperature of 40 °C and the 1st day of drying, the total β-ocimene value was at its highest value to answer the aim of this research, and it decreased insignificantly until the 2nd day of drying, then decreased significantly until the 4th day of drying. At a temperature of 50 °C, the pattern is almost the same as at 40 °C but with a lower value. While at temperatures of 30 and 60 °C, the graph continues to decline until the last day of drying. The increasing pattern at temperatures of 40 and 50 °C was due to the good conditions for protein soluble in the system, the enzymatic reaction proceeds well, and the resulting product can be high. This temperature is also able to restrain the activity of oxidative enzymes, so that the activity of monoterpenoid-synthesizing enzymes can be maximized [16]. While the high temperature and longer drying time, the system condition is no longer good, and productivity decreases.

Conclusions

The conclusion is that the treatments of the variations of drying time and temperature in this study significantly affect every parameter. For the aw value, the increase in treatment significantly decreases the aw value of the material. The total value of soluble protein tended to follow the graph of the pH value of the material when compared with the pattern of changes in the value of aw. As for the enzyme activity of DXS, GPPs and MTPs, the changes were relatively following the graph of total soluble protein. At temperatures of 30 and 60 °C, enzyme activity tended to decrease immediately from the 1st to the last day of drying with a higher intensity at 60 °C. Meanwhile, temperatures of 40 and 50 °C were able to increase the enzyme activity of GPPs and MTPs when compared to DXS at the beginning of the drying process. This is also able to increase the total β-ocimene value at that drying temperature, where the highest total β-ocimene value in this study was at a drying temperature of 40 °C on the 1st day of drying. The conclusions in this study have been obtained and it is hoped that this can be taken into consideration to maintain the quality of Salam leaves during the drying process and the development of research related to this research topic.

Acknowledgements

The authors would like to thank the Ministry of Research and Technology of the Republic of Indonesia as the funder of this research. Likewise, we thank the Faculty of Agricultural Technology, Universitas Gadjah Mada, Indonesia, the advisors who facilitated this research, and everyone involved in the research up to the publication stage.

References

[1] ID Dewijanti, W Mangunwardoyo, N Artanti and M Hanafi. Bioactivities of Salam leaf (Syzygium polyanthum (Wight) Walp). *AIP Conf. Proc*. 2019; **2168**, 020072.

[2] AD Mahmoud, AM Ali, MM Khandaker, HNN Fatihah, NA Awang and N Mat. Discrimination of Syzygium polyanthum cultivars (Wight) Walp based on essential oil composition. *J. Agrobiotech.* 2019; **10**, 1-9.

[3] NM Wartini, PT Ina and GPG Putra. Perbedaan kandungan senyawa volatil daun Salam (Eugenia polyantha Wight) pada beberapa proses curing. *J. Agritech*. 2010; **30**, 231-6.

[4] B Avula, YH Wang, Z Ali, TJ Smillie, V Filion, A Cuerrier, JT Arnason and IA Khan. RP-HPLC determination of phenylalkanoids and monoterpenoids in Rhodiola rosea and identification by LC-ESI-TOF. *Biomed. Chromatogr*. 2010; **24**, 682.

[5] LM Bomfim, LRA Menezes, ACBC Rodrigues, RB Dias, CAG Rocha, MBP Soares, AFS Neto, MP Nascimento, AF Campos, LCRCE Silva, EV Costa and DP Bezerra. Antitumour activity of the microencapsulation of annona vepretorum essential oil. *Basic Clin. Pharmacol. Toxicol*. 2016; **188**, 208-13.

[6] M Sayyah, L Nadjafnia and M Kamalinejad. Anticonvulsant activity and chemical composition of *Artemisia dracunculus* L. essential oil. *J. Ethnopharmacol*. 2004; **94**, 283-7.

[7] J Valente, M Zuzarte, MJ Gonçalves, MC Lopes, C Cavaleiro, L Salgueiro and MT Cruz. Antifungal, antioxidant and anti-inflammatory activities of Oenanthe crocata L. essential oil. *Food Chem. Toxicol*. 2013; **62**, 349-54.

[8] P Cascone, L Iodice, ME Maffei, S Bossi, GI Arimura and E Guerrieri. Tobacco overexpressing β-ocimene induces direct and indirect responses against aphids in receiver tomato plants. *J. Plant Physiol*. 2015; **173**, 28-32.

[9] M Schott, B Klein and A Vilcinskas. Detection of illicit drugs by trained honeybees (*Apis mellifera*). *PLoS One* 2015; **10**, e0128528.

[10] D Tritsch, A Hemmerlin, TJ Bach and M Rohmer. Plant isoprenoid biosynthesis via the MEP pathway: In vivo IPP/DMAPP ratio produced by (E)-4-hydroxy-3-methylbut-2-enyl diphosphate reductase in tobacco BY-2 cell cultures. *FEBS Lett*. 2010; **584**, 129-34.

[11] F Zhou and E Pichersky. More is better: The diversity of terpene metabolism in plants. *Curr. Opin. Plant Biol*. 2020; **55**, 1-10.

[12] C Külheim, SH Yeoh, J Maintz, WJ Foley and GF Moran. Comparative SNP diversity among four *Eucalyptus* species for genes from secondary metabolite biosynthetic pathways. *BMC Genomics* 2009; **10**, 452.

[13] R Zheng, C Liu, Y Wang, J Luo, X Zeng, H Ding, W Xiao, J Gan and C Wang. Expression of mep pathway genes and non-volatile sequestration are associated with circadian rhythm of dominant

terpenoids emission in *Osmanthus fragrans* Lour. flowers. *Front. Plant Sci*. 2017; **8**, 1869.
[14] R Jutamongkon and S Charoenrein. Effect of temperature on the stability of fruit bromelain from smooth cayenne pineapple. *Kasetsart J. Nat. Sci*. 2010; **44**, 943-8.
[15] KS Raja, FS Taip, MMZ Azmi and MRI Shishir. Effect of pre-treatment and different drying methods on the physicochemical properties of *Carica papaya* L. leaf powder. *J. Saudi Soc. Agric. Sci*. 2019; **18**, 150-6.
[16] ZH Loh and YY Lim. Drying effects on antioxidant activity, enzyme activity, and phytochemicals of avocado (Persea americana) leaves. *J. Food Process. Preserv*. 2018; **42**, e13667.
[17] BP Pratama, Supriyadi, RT Swasono and Y Pranoto. Different leaf maturities and withering durations affect the antioxidant potential and aroma compound of Indonesian bay leaf [Syzygium polyanthum (Wight) Walp.]. *Int. Food Res. J*. 2021; **28**, 1196-203.
[18] AR Sloan, ML Dunn, LK Jefferies, OA Pike, SEN Barrows and FM Steele. Effect of water activity and packaging material on the quality of dehydrated taro (*Colocasia esculenta* (L.) Schott) slices during accelerated storage. *Int. J. Food Sci.* 2016; **2016**, 9860139.
[19] FI Abdullah, LS Chua and Z Rahmat. Comparison of protein extraction methods for the leaves of *Ficus deltoidea*. *J. Fundam. Appl. Sci*. 2017; **9**, 908-24.
[20] HU Kosegarten, B Hoffmann and K Mengel. Apoplastic pH and Fe3+ reduction in intact sunflower leaves. *Plant Physiol*. 1999; **121**, 1069-79.
[21] GE Lester, DM Hodges, RD Meyer and KD Munro. Pre-extraction preparation (fresh, frozen, freeze-dried, or acetone powdered) and long-term storage of fruit and vegetable tissues: Effects on antioxidant enzyme activity. *J. Agric. Food Chem*. 2004; **52**, 216-73.
[22] LP Wright and MA Phillips. Measuring the activity of 1-deoxy-D-xylulose 5-phosphate synthase, the first enzyme in the MEP pathway, in plant extracts. *Methods Mol. Biol*. 2014; **1153**, 9-20.
[23] TH Chang, FL Hsieh, TP Ko, KH Teng, PH Liang and AHJ Wang. Structure of a heterotetrameric geranyl pyrophosphate synthase from mint (Mentha piperita) reveals intersubunit regulation. *Plant Cell* 2010; **22**, 454-67.
[24] N Dudareva, D Martin, CM Kish, N Kolosova, N Gorenstein, J Fäldt, B Miller and J Bohlmann. (E)-β-ocimene and myrcene synthase genes of floral scent biosynthesis in snapdragon: Function and expression of three terpene synthase genes of a new terpene synthase subfamily. *Plant Cell* 2003; **15**, 1227-41.
[25] EJ Gomez, JA Delgado and JM Gonzalez. Influence of water availability and temperature on estimates of microbial extracellular enzyme activity. *PeerJ* 2021; **9**, e10994.
[26] S Mujaffar and S Bynoe. Investigations into hot air and microwave drying of the west. *In*: Proceedings of the 7th European Drying Conference, Torino, Itali. 2019, p. 23.
[27] TP Labuza, ND Heidelbaugh, M Silver and M Karel. Oxidation at intermediate moisture contents. *J. Am. Oil Chem. Soc*. 1971; **48**, 86-90.
[28] Z Ai, B Zhang, Y Chen, Z Yu, H Chen and D Ni. Impact of light irradiation on black tea quality during withering. *J. Food Sci. Technol*. 2017; **54**, 1212-27.
[29] RF Mechlouch, W Elfalleh, M Ziadi, H Hannachi, M Chwikhi, AB Aoun, I Elakesh and F Cheour. Effect of different drying methods on the physico-chemical properties of tomato variety 'Rio Grande'. *Int. J. Food Eng*. 2012; **8**, 4.
[30] AB Ninfa. *Fundamental laboratory approaches for biochemistry and biotechnology*. 2nd eds. John Wiley, Hoboken, New Jersey, 2010.
[31] LCAD Silva, TL Honorato, RS Cavalcante, TT Franco and S Rodrigues. Effect of pH and temperature on enzyme activity of chitosanase produced under solid stated fermentation by Trichoderma spp. *Indian J. Microbiol*. 2012; **52**, 60-5.
[32] S Handa, D Ramamoorthy, TJ Spradling, WC Guida, JH Adams, KG Bendinskas and DJ Merkler. Production of recombinant 1-deoxy-d-xylulose-5-phosphate synthase from Plasmodium vivax in Escherichia coli. *FEBS Open Bio* 2013; **3**, 124-9.
[33] ZA Demissie, LAE Erland, MR Rheault and SS Mahmoud. The biosynthetic origin of irregular monoterpenes in Lavandula: Isolation and biochemical characterization of a novel cis-prenyl diphosphate synthase gene, lavandulyl diphosphate synthase. *J. Biol. Chem*. 2013; **288**, 6333-41.
[34] D Andres-Montaner. 2008, Study of gene regulation and function of monoterpene synthases in Holm oak (*Quercus ilex* L.) and transgenic birch (*Betula pendula* Roth) (*in German*). Ph. D. Thesis. Albert Ludwig University of Freiburg, Freiburg, Germany.
[35] R Rehman, MA Hanif, Z Mushtaq and AM Al-Sadi. Biosynthesis of essential oils in aromatic plants. *Food Rev. Int*. 2016; **32**, 117-60.