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The Effect of Drying Time on the Antioxidant Properties of Single Black Garlic Powder

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Abstract: Black garlic is a processed product derived from fresh garlic that generally has a taste and aroma that is less preferred. A drying process is employed to increase the attractiveness of consuming black garlic. This study aims to assess the impact of the drying time on the antioxidant activity and content of antioxidant compounds such as phenolics, flavonoids, and tannins in single black garlic. The drying time is expected to yield a single black garlic powder that is more convenient for consumption, whether in capsule form or as an addition to food products. With low moisture content, single black garlic powder has a longer shelf life and high antioxidant activity, potentially commanding a higher market value. In this research, the drying time was carried out on a single black garlic using an oven for 6, 12, 18, and 24 hours. The analysis of the total phenolic content (TPC), entire tannin content (TTC), and total flavonoid content (TFC) was determined using a Uv-Vis spectrophotometer, and each component was measured concerning gallic acid equivalent for TPC, quercetin for TFC, and tannic acid for TTC. Antioxidant activity was determined using the DPPH method, and the outcomes were expressed as IC_{50} values. The research shows that the extended drying time leads to an augmentation of the antioxidant compound in black garlic. Drying for 24 hours produces significant outcomes, showing potent antioxidant activity with an IC_{50} value of 37,019 ppm (powerful antioxidant category), total phenolic content value is 44,785 ± 0,033 mgGAE/g, total flavonoid content value is 32,033 ± 0,008 mgQE/g, and total tannins content value is 74,884 ± 0,057 mgTAE/g. Statistical analysis using a one-way ANOVA showed a significant influence of drying time on TPC, TFC, and TTC ($p < 0,05$). The longer the drying time, the higher the antioxidant activity and the content of compounds acting as antioxidants in black garlic powder.

Keywords: Antioxidant; Black Garlic Powder; Flavonoids; Phenolic; Tannins.

Introduction

Garlic is widely used in the fields of food and health. However, fresh garlic as an herbal remedy is still limited due to its strong aroma and taste, which can cause digestive disturbances in some individuals [1]. Therefore, processed garlic products, including black garlic, have been developed. Black garlic is produced by heating fresh garlic at a specific temperature and time [2]. During the heating process, characteristic changes occur in garlic, resulting in black garlic with its distinctive color, sweet taste, and chewy texture [3].

The reduction of allicin content influences the decrease in taste intensity and unwanted aroma in black garlic [4]. Several studies have reported changes in black garlic's nutritional components, bioactive compounds, and antioxidant activities during heating. The enhanced antioxidant activity of black garlic is attributed to the increased presence of *S-allyl cysteine* (SAC) and phenolic compounds, such as flavonoids and tannins [5]. Phenolic compounds are organic compounds containing hydroxyl groups (-OH) attached to carbon atoms in aromatic rings. As antioxidants, phenolic compounds donate hydrogen atoms, reducing free radicals into a more stable form [6]. The phenolic content of black garlic heated for 21 days is reported to be 52,325 mg GAE/g, total flavonoid content at 339,875 mg QE/g, total tannin content at 553,165 mg TAE/g and antioxidant activity indicated by an IC_{50} value of 58,5 ppm, [7].

Black garlic has also been proven to have health benefits; the health advantages of black garlic have been demonstrated, including its ability to inhibit the growth of cancer cells, anti-allergic effects, prevent premature aging, anti-obesity properties, and lower blood sugar levels [8]. Therefore, regular consumption of black garlic can provide sound health effects. The previous research on the evaluation of consumer purchasing interest in black garlic stated that only 28% of consumers expressed a willingness to buy black garlic. In contrast, others answered uncertainly or said they would not purchase black garlic due to low preference levels [9]. Therefore, further processing of black garlic is needed to improve its attractiveness to consumers.

Processing black garlic into powder can be an innovation that positively impacts society, especially regarding health. Black garlic powder can be packaged in capsule form, and it is expected to increase consumer interest because it is easier to consume. In addition, the low moisture content in black garlic extends its shelf life, so powdered black garlic often has a higher market value [10]. Black garlic powder with a higher market value should ideally contain higher levels of beneficial compound components, such as antioxidant compounds. Phenolic compounds like flavonoids and tannins can act as natural antioxidants. The process of turning black garlic into powder can be achieved through a drying process. Therefore, this study investigates the effect of drying time on the antioxidant activity and compounds acting as

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antioxidants, such as phenolic compounds, flavonoids, and tannins, in single black garlic powder.

Increasing temperature and drying duration can enhance antioxidant activity but may also damage compounds acting as antioxidants in black garlic. Hence, optimization is necessary. A study by Yuniar (2018) indicated the influence of drying duration (15, 16, and 17 hours) at 80°C on antioxidant activity and phenolic compound content in black garlic powder. Drying for 15 hours resulted in antioxidant activity with an IC_{50} of 709,70 ppm and total phenolic compounds of 7,33 ± 0,09 mg GAE/g. Drying for 17 hours produced the highest antioxidant activity with an IC_{50} value of 579,48 ppm and total phenolic compounds of 11,10 ± 0,48 mg GAE/g. This demonstrates increased antioxidant activity and phenolic compound content in black garlic with longer drying durations. In this study, drying was conducted using an oven for 6, 12, 18, and 24 hours at 80°C. The use of 80°C temperature, as per research by Herdyastuti (2021), showed an increase in total phenolic content (21,0982 ± 0,089 mg GAE/g) and optimum antioxidant activity (146,2954 ppm). Therefore, further research is needed on the effect of drying duration on antioxidant activity and compounds acting as antioxidants, such as phenolic compounds, flavonoids, and tannins, in single black garlic powder at 80°C for 6, 12, 18, and 24 hours, as it is not yet well understood and studied.

Research Methods

This research uses the true experimental method. Some of the tools used in this study are oven (Daihan Labtech), blender (Oxone), 60-mesh sieve, analytical balance (Denver Instrument SI-234), rotary evaporator (Buchi), micropipette (Minipcr), glassware (Iwaki Pyrex), vial bottles, UV-Vis Spectrophotometer (Shimadzu UV-1800), vortex mixer (Labnet VX-200).

The materials used in this study were single black garlic obtained from a distributor in Malang, distilled water, ethanol 96% (Smart-Lab), methanol p.a 99,9% (Smart-Lab), DPPH solution (Sigma Aldrich), Folin-Ciocalteu 10% (Merck), Na_2CO_3 7,5% (Merck), gallic acid (Merck), quercetin (Sigma Aldrich), $AlCl_3$ 10% (Merck), tannic acid (Merck).

Samples were prepared by slicing black garlic into 4-5 mm and dried in an oven for 6, 12, 18, and 24 hours at 80 °C. Control samples (single fresh garlic and single black garlic without the drying process) were also prepared. After that, they were ground using a blender. 50 g of each sample, maceration using a 1:5 ratio of 96% ethanol for 48 hours at room temperature. The macerated samples were filtered using a Buchner funnel. The obtained filtrate was evaporated using a vacuum rotary evaporator at a temperature of ± 40 °C [11].

Total phenolic content (TPC) is analyzed using the colorimetric assay with gallic acid as its standard for the calibration curve. 0,5 mL of an extract of 1000 ppm, 1,5 mL of Folin-Ciocalteu 10%, and 1,2 mL of 7,5% Na_2CO_3 were added to the test tube. The mixture is vortexed for 10 seconds and left to stand for 30 minutes at room temperature in the dark. The absorbance of the solution was determined using a spectrophotometer Uv-Vis at 740,5 nm. The TPC value is expressed as mg gallic acid equivalent per gram of extract (mgGAE/g) [12].

Total flavonoid content (TFC) is analyzed using the colorimetric assay with quercetin as its standard for the calibration curve [13]. 0,5 mL of extract 1000 ppm, 1,5 mL ethanol p.a, 0,1 mL $AlCl_3$ 10%, 0,1 mL CH_3COOK 1M, and 2,8 mL distilled water were added to the test tube. The mixture is vortexed for 10 seconds and left to stand for 30 minutes at room temperature in the dark. The absorbance of the solution was determined using a spectrophotometer UV-Vis at 440,10 nm. The TPC value is expressed as mg quercetin equivalent per gram of extract (mgQE/g) [14].

Total tannin content (TTC) was analyzed using the colorimetric assay with tannic acid as the standard for the calibration curve. 1 mL of an extract of 1000 ppm, 1 mL of Folin-Ciocalteu 10%, and 1 mL of 20% Na_2CO_3 were added to the test tube. The mixture is vortexed for 10 seconds and left to stand for 30 minutes at room temperature in the dark. The absorbance of the solution was determined using a spectrophotometer Uv-Vis at 747 nm. The TTC value is expressed as mg tannic acid equivalent per gram of extract (mgTAE/g) [15].

The antioxidant activity is analyzed using the DPPH assay. 2 mL of extract 1000 ppm and 1 mL of 0,004% DPPH solution were added to the test tube. The mixture is vortexed for 10 seconds and left to stand for 30 minutes at room temperature in the dark. Pippete 2 mL of methanol p.a, add 1 mL of DPPH solution, and vortex for 1 minute to make the blank solution. The absorbance of the solution was determined using a spectrophotometer Uv-Vis at 514,6 nm. The absorbance data obtained calculates the percentage of inhibition (%I) with the equation: %I = (A - B / A) x 100%, where A = absorbance of the control solution; B = absorbance of the test solution. Next, a graph is constructed between the percentage of I and the sample concentration to obtain a regression equation to determine the IC_{50} value of the sample extract [16]. The strength of antioxidant activity can be categorized into three levels: very strong (<50 ppm), strong (50-100 ppm), moderate (101-150 ppm), weak (151-200 ppm), and very weak (>200 ppm) [17].

Results And Discussion

Single-dried black garlic and control samples are shown in Figure 1. It shows that the dried black garlic sample changes color and texture. The dried black garlic exhibits a firm texture, signifying a reduction in moisture content due to the drying process. A concentrated yellow extract is obtained from the single garlic sample through maceration. In contrast, a dark brown extract is obtained from the single black garlic and the dried single black garlic samples (Figure 2). The color variations observed in the samples are attributed to the Maillard reaction between reducing sugars and amino acids during heating. The Maillard reaction forms melanoidin compounds, changing the color from garlic to a dark brown shade. The longer the heating process, the darker the color produced. The heating process also reduces the pungent aroma of black garlic due to the decrease in allicin content, which transforms into the antioxidant compounds S-allyl cysteine (SAC) and polyphenolic compounds [3].

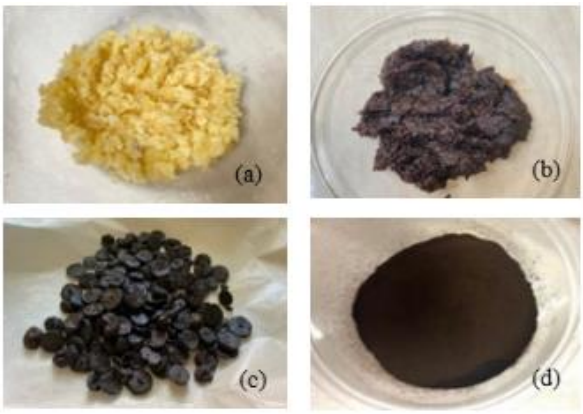

Figure 1. Samples used: (a) Single white garlic, (b) single black garlic, (c) dried black garlic, (d) black garlic powder

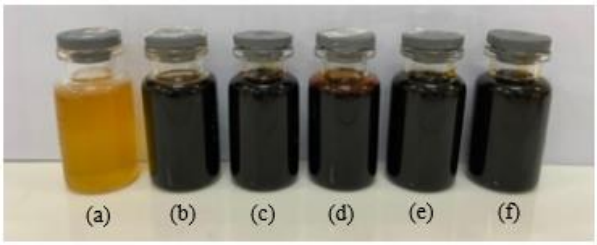

Figure 2. Concentrated extract samples (a) single white garlic; (b) single black garlic; (c) dried black garlic for 6 hours; (d) dried black garlic for 12 hours; (e) dried black garlic for 18 hours; (f) dried black garlic for 24 hours

Total Phenolic Content (TPC)

The control samples and dried black garlic samples have the potential as antioxidants. The analysis results indicated that drying time affects the TPC in black garlic ($p < 0.05$), as shown in Table 1. The data showed that the TPC of the control sample significantly differed compared to dried black garlic. Black garlic dried for 24 hours yielded the highest TPC, 44,785 ± 0,033 mgGAE/g.

Table 1. TPC Result

Sample	TPC (mg GAE/g) ± SD
Single garlic	2.764 ± 0.051
Single black garlic	4.111 ± 0.031
Dried black garlic for 6 hours	23.163 ± 0.042[a]
Dried black garlic for 12 hours	31.573 ± 0.010[b]
Dried black garlic for 18 hours	36.201 ± 0.033[c]
Dried black garlic for 24 hours	44.785 ± 0.033[d]

*Notes: Numbers followed by different letters indicate a significant difference at the same level within a column based on the Duncan post hoc test (Sig < 0.05).

The increase in TPC is due to the drying process, which causes damage to the cell wall components, releasing phenolic compounds from insoluble components such as proteins. This results in an elevated amount of phenolic compounds obtained during extraction [18]. The drying process also reduces water content, increasing the total solids in the powder. Total solids are carbohydrates, proteins, fats, vitamins, and minerals that undergo degradation [19]. These components influence the total solids produced because many antioxidant compounds, such as phenolic compounds, bind with other elements like fiber and protein. This analysis result is consistent with the previous research, which indicates that the total phenolic content increases with the prolonged drying time. The highest total phenolic content was observed in black garlic samples dried for 17 hours, yielding a value of 22.5 mg GAE/g extract [10].

Antioxidant Activity

The results of antioxidant activity analysis on the samples are presented as IC_{50} values in Table 2. The IC_{50} value is inversely proportional to antioxidant capacity; the smaller the IC_{50} value, the higher the antioxidant activity in the sample. Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-1picrylhidrazyl) method. This method depends on the reduction reaction of the unpaired electron on the nitrogen atom, facilitated by a hydrogen atom derived from the antioxidant. This process results in the formation of a hydrazine group. As a result, the DPPH structure stabilizes, forming the yellow-colored compound DPPH-H (Figure 3). In this method, the changes in DPPH absorption can be measured, indicating the unreacted DPPH remaining after reacting with the antioxidant [20].

Table 2. IC_{50} value

Sample	IC_{50} (ppm)	Antioxidant categories
Single garlic	280.439	Very weak
Single black garlic	298.716	Very weak
Dried black garlic for 6 hours	64.936	Strong
Dried black garlic for 12 hours	55.636	Strong
Dried black garlic for 12 hours	48.908	Very strong
Dried black garlic for 12 hours	37.019	Very strong

Based on Table 4, the drying time influences the antioxidant activity values of a single black garlic powder. There is a significant decrease in the IC_{50} value in the dried black garlic powder samples. Drying the black garlic for 6 hours resulted in an IC_{50} value of 64,936 ppm, which decreased with increasing drying time. The highest antioxidant activity was obtained in the single black garlic sample dried for 24 hours, with an IC_{50} value of 37,019 ppm. Based on the categories of antioxidant activity, samples of single black garlic dried for 6 and 12 hours are categorized as strong antioxidants, while samples dried for 18 and 24 hours are classified as powerful antioxidants.

The increase in antioxidant activity in the dried black garlic samples may be attributed to the drying process, which releases phenolic compounds, thereby increasing the amount of active compounds in the sample. Phenolic compounds such as flavonoids and tannins are believed to exhibit antioxidant activity due to the presence of hydroxyl groups capable of donating hydrogen atoms to free radicals [21]. This is consistent with research conducted by Mayulu & Sawitri (2023), which stated that the heating process in black garlic produces tannin compounds at 553,165 mg Tannic acid/g and flavonoid compounds at 51,325 mg QE/g, which are suspected to act as antioxidant sources in black garlic.

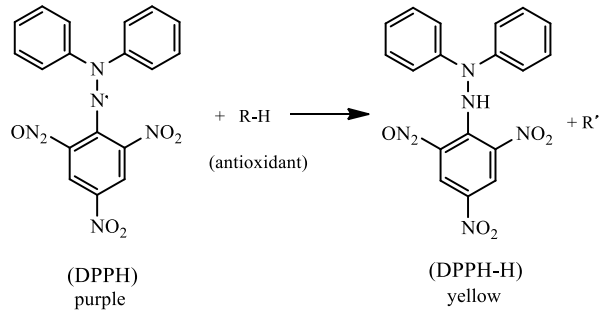

Figure 3. Reaction of DPPH with antioxidant compound

Phenolic compounds as antioxidants can stabilize free radicals by releasing hydrogen atoms through electron transfer mechanisms, transforming phenol into phenoxyl radicals. Through resonance effects, phenoxyl radicals can undergo stabilization. This property makes derivatives of phenol effective hydrogen donors in inhibiting reactions caused by radical compounds. The scavenging activity of phenolic compounds against free radicals is affected by the quantity of phenolic hydrogen positioned within their molecular structure. The higher the hydroxyl groups in phenolic compounds, the greater the antioxidant activity produced [22].

Total Flavonoid Content (TFC)

The total flavonoid content was determined through UV-Vis spectrophotometry using the colorimetric ($AlCl_3$) method, with quercetin serving as a standard for the calibration curve. The reaction of the $AlCl_3$ reagent with quercetin can be observed in Figure 3. The principle of flavonoid determination with the $AlCl_3$ colorimetric method involves the formation of a colored complex with aluminum chloride ($AlCl_3$) with keto groups at the C-4 atom and hydroxy groups at the C-3 or C-5 atoms grouped into flavones and flavonols [23]. The antioxidant properties of flavonoids come from their capacity to contribute hydrogen atoms or their ability to chelate metals [24].

Table 2. TFC Result

Sample	TFC (mg QE/g) ± SD
Single garlic	6.281 ± 0.157
Single black garlic	5.114 ± 0.049
Dried black garlic for 6 hours	15.229 ± 0.049[a]
Dried black garlic for 12 hours	22.562 ± 0.044[b]
Dried black garlic for 18 hours	23.986 ± 0.038[c]
Dried black garlic for 24 hours	32.033 ± 0.008[d]

*Notes: Numbers followed by different letters indicate a significant difference at the same level within a column based on the Duncan post hoc test (Sig < 0.05).

The analysis of TFC of the control and dried black garlic samples indicates an increase in TFC in the dried black garlic sample. One-way ANOVA statistical analysis shows a significant influence on dried black garlic ($p < 0,05$), as shown in Table 2. The data showed that the TFC of the control sample significantly differed compared to dried black garlic. Black garlic dried for 24 hours yielded the highest TFC, 32,033 ± 0,008 mgQE/g. The drying process causes the release of antioxidant components due to cell wall damage caused by heat or macro-wave effects, facilitating the release and extraction of phenolic compounds. During drying, many flavonoid compounds are released and bound to other compounds through ester or glycoside bonds. The result leads to an increase in flavonoid levels as the drying time increases. Previous research indicates that drying for 15 hours resulted in a total flavonoid content of 1,40 ± 0,09 mgQE/g extract, which increased to 2,78 ± 0,14 mgQE/g extract after drying for 17 hours [10].

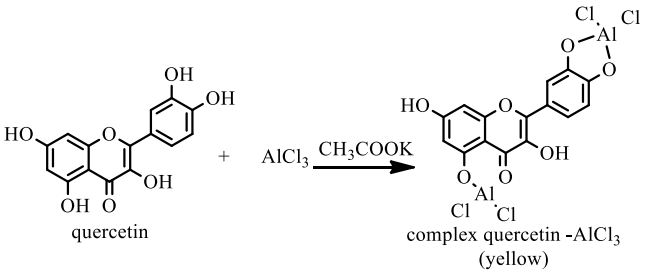

Figure 3. Mechanism of complex quercetin- $AlCl_3$

Total Tannins Content (TTC)

The determination of total flavonoid levels is conducted according to several studies, indicating that tannins play a role as antioxidant compounds. Based on this study, the black garlic and dried black garlic samples have the potential as antioxidants. The analysis results indicated that drying time affects the TTC in black garlic ($p < 0.05$), as shown in Table 3. The results indicated a significant disparity in the TTC between the control sample and dried black garlic. Black garlic dried for 24 hours yielded the highest TTC, 74,884 ± 0,054 mgTAE/g.

Table 3. TFC Result

Sample	TTC (mg TAE/g) ± SD
Single garlic	0.752 ± 0.142
Single black garlic	11.858 ± 0.142
Dried black garlic for 6 hours	61.560 ± 0.142[a]
Dried black garlic for 12 hours	66.449 ± 0.022[b]
Dried black garlic for 18 hours	72.426 ± 0.046[c]
Dried black garlic for 24 hours	74.884 ± 0.054[d]

*Notes: Numbers followed by different letters indicate a significant difference at the same level within a column based on the Duncan post hoc test (Sig < 0.05).

The drying process significantly influences the content of active plant compounds, including tannins [25]. The increase in tannin content in black garlic is attributed to the drying process, which is believed to deactivate catechol oxidase enzymes, thus allowing tannin compounds to remain intact and protected within the plant tissue structure, resulting in high tannin levels [26]. Catechol oxidase is considered one of the enzymes responsible for browning reactions in food because it accelerates the oxidation and degradation of polyphenols and their derivatives [27]. According to the research findings, the total tannin content increases with longer drying times. These findings are consistent with a study conducted by Hutasoit et al. (2021), which stated that drying time affects the increased tannin content, with the highest value recorded at 168.30 mg TAE/g extract after drying for 7 hours.

Correlation between antioxidant activity and the TPC, TFC, and TTC

Phenolic compounds are crucial plant components for scavenging free radicals due to the role of their hydroxyl groups. Flavonoids and tannins are the largest groups of phenolic compounds and possess antioxidant capabilities [28]. The correlation between antioxidant activity and TPC, TFC, and TTC is shown in Figure 4.

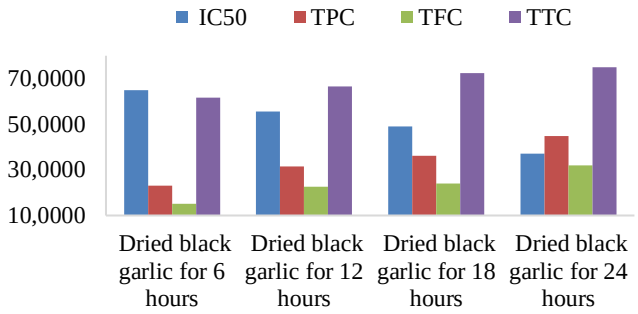

Figure 4. IC_{50}, TPC, TFC, TTC result

Based on Figure 4, the graph illustrates an increase in total phenolic, flavonoid, and tannin levels in dried single black garlic samples, correlating with the prolonged drying time. Antioxidant activity is expressed in IC_{50} values. The relationship between IC_{50} values and antioxidant activity is inverse, where lower IC_{50} values indicate higher antioxidant activity. Therefore, it can be concluded that the content of phenolic compounds, flavonoids, and tannins influences the antioxidant activity of dried black garlic. The higher the concentration of phenolic compounds, flavonoids, and tannins, the higher the antioxidant activity. That is consistent with the research conducted by Mayulu & Sawitri (2023), which mentions increased antioxidant activity, total phenolics, flavonoids, and tannins in heated black garlic, along with more extended time used.

Conclusion

Based on this study, the drying time treatment significantly influences the production of higher antioxidant activity, total phenolics, flavonoids, and tannins in black garlic powder. With prolonged drying, the antioxidant activity and the presence of compounds acting as antioxidants in black garlic powder increase. Drying for 24 hours yields the highest results with an IC_{50} antioxidant activity value of 37,019 ppm, total phenolic content of 44,785 ± 0,033 mgGAE/g extract, total flavonoid content of 32,033 ± 0,008 mgQE/g extract, and total tannin content of 74,884 ± 0,054 mgTAE/g extract. This indicates that the drying process in single black garlic can enhance antioxidant activity. Further research is needed to utilize single black garlic powder in capsule form, which can be consumed directly, and as an additive in food in powdered form.

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