



RESEARCH ARTICLE

# Bulb maturity and storage effect on total phenolic and flavonoid contents and antioxidant activity of freeze-dried garlic

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## Abstract

A study on the morphological characterization of one local garlic variety was conducted. The effects of various maturity levels and storage were evaluated via measurement of parameters such as Total Phenolic Content (TPC), Total Flavonoid Contents (TFC) and antioxidant activity. The assessments were done in plants divided into four groups depending upon the period of maturity i.e. 90, 100, 110 and 120 Days After Planting (DAP) and three storage periods i.e. 30, 60 and 90 days under ambient conditions. Results showed that bulb weight, bulb diameter and clove weight increased as the garlic matured. These morphological characteristics showed highest values at maturity achieved at 120 DAP. Before storage, TPC and TFC were not affected by maturity, irrespective of the group. The antioxidant activity was higher at 90 and 110 DAP. After storage, maturity group and storage period showed a significant effect on the TPC, TFC and antioxidant activity. TPC was significantly decreased after storage as compared to without storage. Values of the parameters were highest at maturity achieved at 100 and 120 DAP. Whereas storage exhibited a decline in contents across maturity groups in the case of TFC. In 90 to 120 DAP, a decreasing trend of the TFC was observed from 30 to 90 days of storage. A 120 DAP maturity and storage for 90 days is recommended for the farmers to obtain bigger bulbs and cloves due to its higher weight and higher TPC and TFC and comparable antioxidant activity with the other maturity groups. Analysis of other secondary metabolites is recommended.

**Keywords:** *Allium sativum*; freeze-drying of garlic; garlic antioxidants; garlic pungency storage; maturity

## Introduction

Garlic (*Allium sativum* L.) is a perennial herb of the Amaryllidaceae or Alliaceae family. Garlic is a widely used bulbous vegetable owing to its pungent flavor and potential health benefits due to bioactive compounds, such as allicin, a sulfur-containing volatile compound (1). Garlic is a cash crop of Ilocos Norte, Philippines, which is the top producer of garlic. The garlic production from this region of the Philippines contributes more than 65 % to the country's total production. This is because the region shows suitability for garlic cultivation (2). The garlic production is affected by complex factors such as climatic conditions and maturity, or the harvesting time. In this study, the maturity is considered, which affects the morphological characteristics (bulb weight, clove count per bulb and clove weight).

Garlic is a good source of diverse bioactive compounds, such as phenolics, flavonoids, organic sulfides and saponins (3). However, the amount of phenolic compounds is affected by various factors, including the maturity or harvesting time (4,5). Harvesting time has a significant impact on the phenolic and flavonoid contents. It was reported that late harvesting had lower concentrations of phenolic and flavonoid contents (5). However, in a previous study, irrespective of the harvesting time, the total polyphenols were not affected, but

the specific phenolic compounds, such as phenolic acids and flavonoids, were affected by harvesting time. Therefore, a preservation is needed to obtain a reasonable amount of phenolic compounds and flavonoids. The increase in the amount of phenolic acids and flavonoids with the maturity of bulbs was noted (6). The highest amounts were observed at the later stage.

Freeze-drying is one of the best methods of food preservation. Freeze-drying or lyophilization is done under low temperature and high vacuum (7). It helps in preserving the flavor, aroma and nutritional value of food items. Reports have suggested that freeze-drying of garlic cloves did not affect the polyphenol content and antioxidant activity as compared to unpreserved cloves (8). Studies suggest that low temperature (4 °C) storage plays an essential role in maintaining the bioactive compounds. Levels of polyphenols, flavonoids and antioxidant activity were not affected by storage time in garlic under It was previously reported (9). As per literature reports, no previous study related to the study of the effect of ambient storage on the phenolic and flavonoid contents and antioxidant activity of the freeze-dried garlic powder, especially for the Ilocos White variety, has been conducted. Thus, the present study was conducted to determine the change in the status of phytochemicals of garlic after storage.

## Materials and Methods

### Sample preparation

Garlic bulbs (Ilocos White variety) were purchased from the local producer (a farmer-cooperator) in Ilocos Norte. Different groups based on the maturity (90, 100, 110 and 120 DAP) of garlic bulbs were assessed. The average bulb maturity being used in the locality is 100 and 110 DAP. The morphological parameters were measured. Later, the garlic bulbs were freeze-dried and converted into powder form. Freeze-dried garlic powder was stored under ambient conditions for 30, 60 and 90 days. Ilocos White was the variety used in the study because of its pungency.

### Bulb weight and diameter

Mature bulbs were weighed individually using a digital weighing scale. A vernier caliper, however, was used to measure the diameter of the bulbs. The outer skins were removed before weighing.

### Clove count per bulb

The outer and inner cloves were counted separately for each mature bulb. The sum of outer and inner cloves was considered for the total clove count.

### Moisture content

Garlic cloves were peeled before oven drying at 105 °C. Drying was done until the weight was stable.

### Total phenolic and flavonoid contents and antioxidant activity

The method adapted from a previous study was used to analyse the total phenolic and flavonoid contents and antioxidant activity of garlic samples. The antioxidant activity was expressed as a percentage (10).

### Statistical analysis

The data obtained was analysed by using the completely randomized design with four replications. The STAR program was used to analyse the data. Treatment means were compared using the Least Significant Difference (LSD) test.

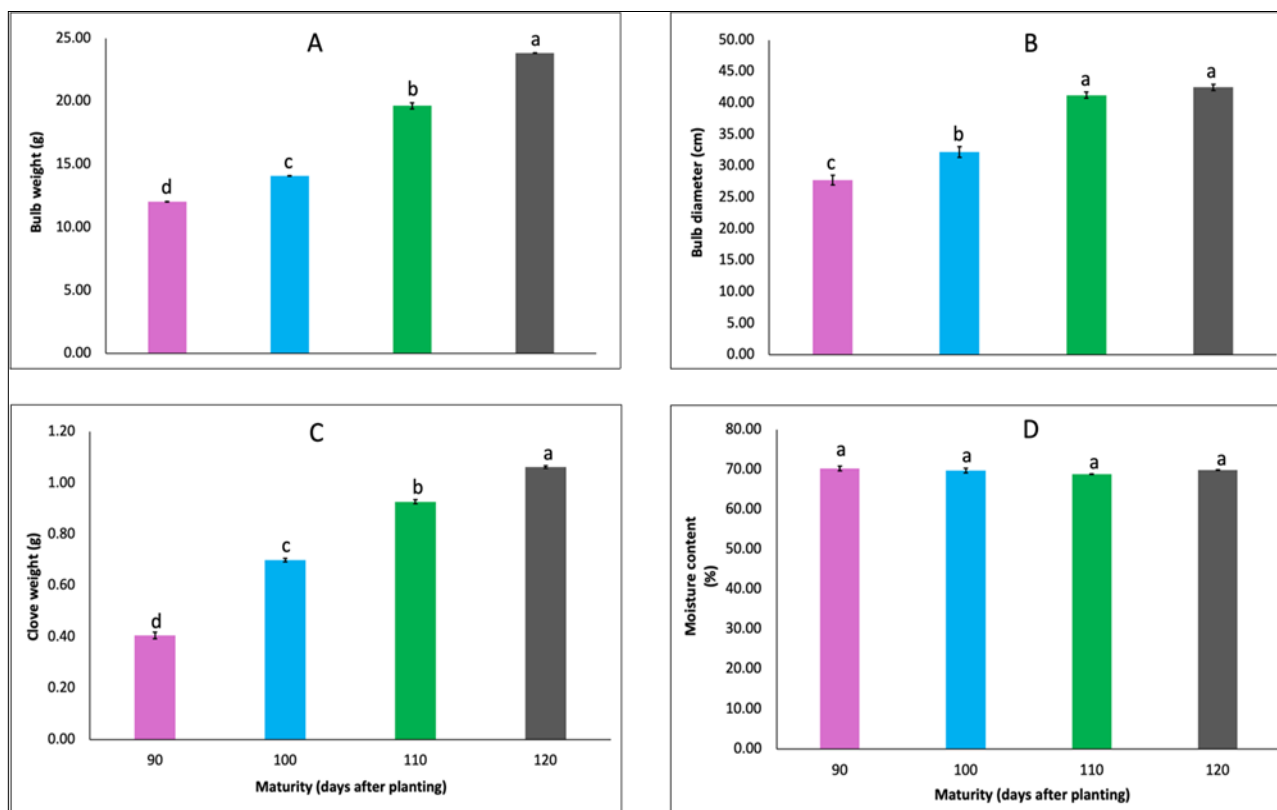
## Results and Discussion

### Bulb weight and diameter

The bulb weight increased at maturity (Fig. 1A). The highest bulb weight was noted after 120 DAP, whereas the lightest bulb weight was observed in 90 DAP. A similar trend was also noted in bulb diameter (Fig. 1B). Similar observations have been reported in a previous study that longer maturity produced heavier bulbs than shorter maturity (11). The studies indicated that as the garlic matures, the absorption of nutrients and moisture from the soil increases. Accordingly, the accumulation of carbohydrates and the biomass increase during the bulb development from 90 to 120 DAP.

### Clove weight and moisture content

A heavier clove weight was obtained when late harvesting was done (Fig. 1C). An increasing trend of clove weight was evident in mature bulbs. The lightest clove weight was obtained from 90 DAP. It indicates that heavier cloves were produced at a later stage of harvesting. However, the moisture content was not affected by maturity. This indicates that, regardless of the maturity between 90 and 120 DAP, the moisture levels were similar.



**Fig. 1.** Bulb characteristics of garlic at different times of maturity.

(A) Comparative bulb weight of garlic; (B) Bulb diameter variations of garlic; (C) Clove weight differences of garlic based on the maturity; (D) Moisture content of cloves

Bars with different letters showed a significant difference at 5 % level using the LSD test

### Clove count per bulb

Samples collected at 120 DAP produced higher outer cloves and high inner cloves were noted in groups after 90 DAP (Fig. 2A, B). However, the inner cloves were higher in 90 DAP than the other maturity groups. The total clove count per bulb was higher in 120 DAP as compared to 100 and 110 DAP. The former showed similarity with 90 DAP (Fig. 2C). Results signify that a lower inner clove means bigger sizes of outer cloves, which can be observed in 120 DAP. Whereas a higher inner cloves per bulb, as observed in 90 DAP, may be due to the maturity which needs longer maturation to develop more outer cloves.

### Total phenolic and flavonoid contents and antioxidant activity

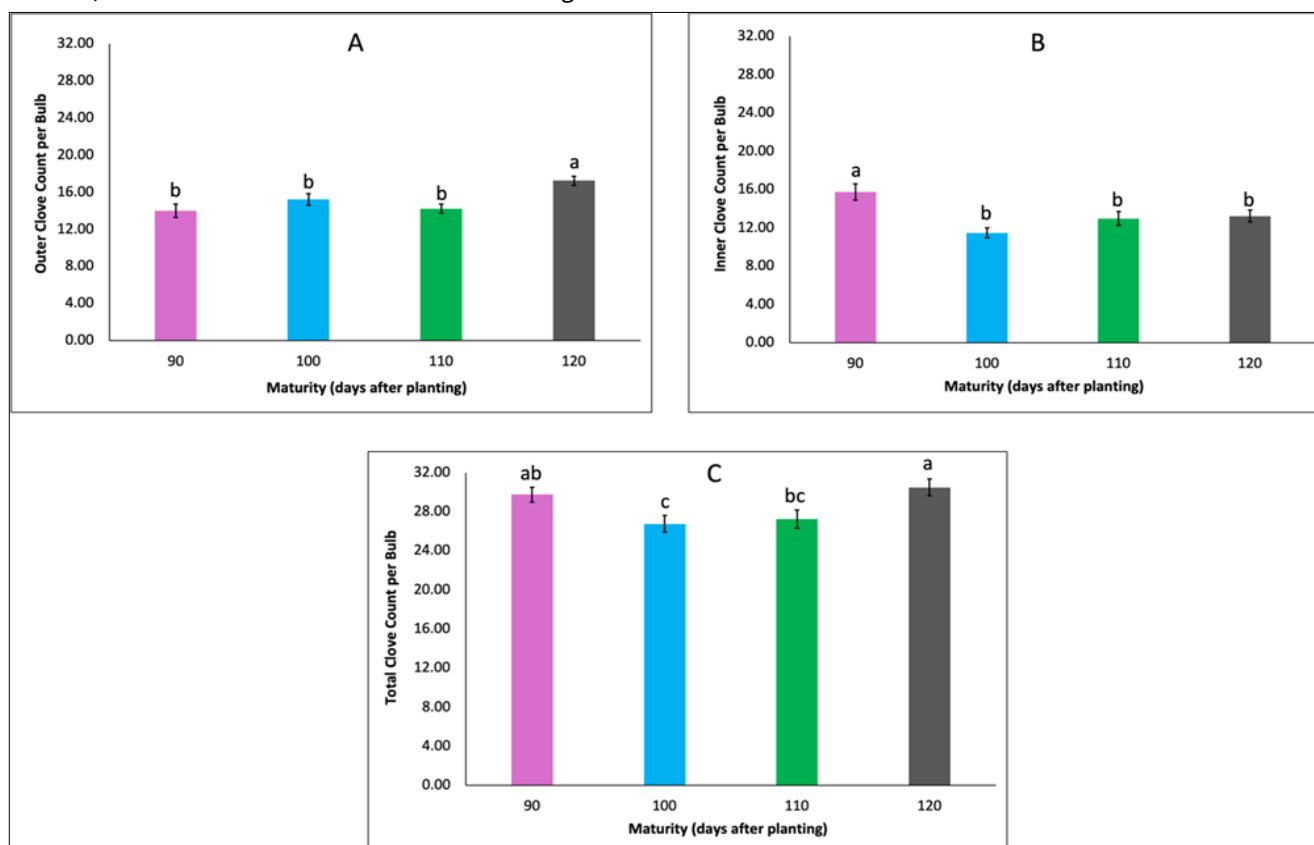
TPC and TFC and antioxidant activity showed a significant variation among storage periods (Fig. 3). The TPC and TFC of garlic were not affected by maturity. However, the antioxidant activity was significantly different among maturity groups (Fig. 3A). Maturity groups, such as 90 and 110 DAP, displayed a higher antioxidant activity than the other maturity groups. The fluctuation may be due to the function of the antioxidants, such as phenolic and flavonoids. However, other antioxidants must be considered for further investigation.

The TPC declined as the storage progressed, except for 120 DAP (Fig. 3B). An abrupt decrease in TPC was observed at 110 DAP after 60 days of storage. At the same storage period, 90 DAP exhibited a slight increase in TPC. Moreover, TPC of 120 DAP was increased after 60 days of storage, which was higher than the other maturity times. A maturity of 110 DAP displayed the lowest TPC after 90 days of storage, followed by 90 and 100 DAP. Likewise, the TPC of 120 DAP was almost two times higher than

110 DAP. It should be noted that flavonoids belong to TPC, which is a large group of secondary metabolites that play a pivotal role as antioxidants (6).

A reduction in TFC was observed after 30 days of storage, affecting all different times of maturity. A gradual decrease in the TFC at different times of maturity after 120 days of storage was observed (Fig. 3C). But a significant increase was observed after 90 days of storage in 120 DAP. The decrease of the TFC during storage may be due to the function of this secondary metabolite as an antioxidant to scavenge free radicals during storage, which was evident in 30 days of storage and in 90 DAP, where there was a continuous decline of TFC throughout the storage. Moreover, the TFC was changing throughout the storage. Earlier studies reported that storage at 20 °C did not affect TFC when storage was done up to 16 weeks (12). A previous study reported that there was a decrease and eventually an increase in flavonoids of shallot bulbs during storage (13), as was also observed in the study. The results indicated that storage had a significant impact on TPC and TFC and it was previously reported that these are dependent on the storage conditions and processing methods of garlic (14,15). Oxidative degradation during storage leads to a decline in levels of phenolic compounds (16). In the present study, a decline in TPC and TFC was noted during the storage periods of 60 and 90 days.

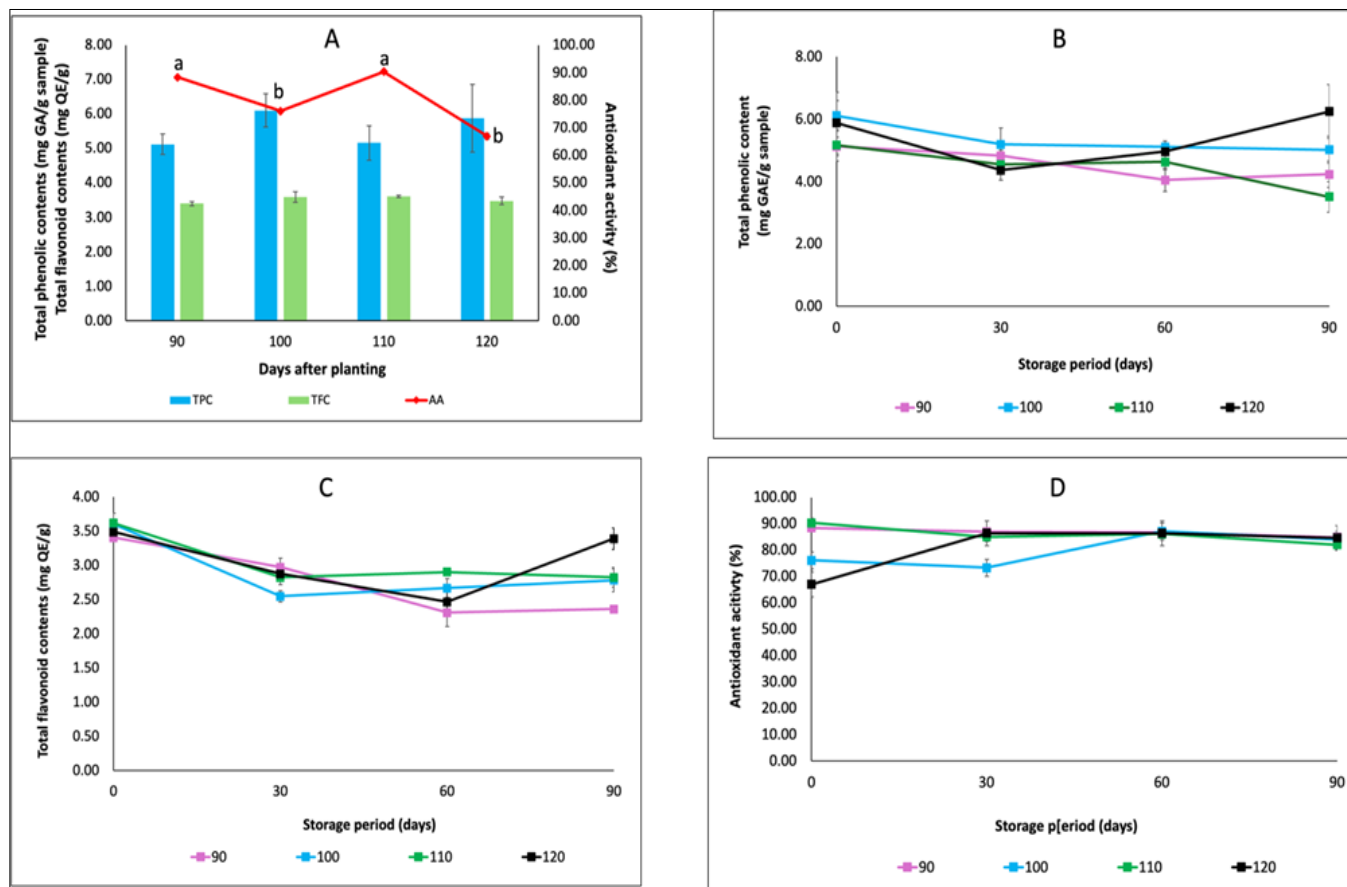
At 30 days of storage period, 90 DAP had a higher antioxidant activity than 100 and 110 DAP, but like 120 DAP (Fig. 3D). Whereas after 60 days of storage, there were no significant differences among maturity groups. A fluctuating antioxidant activity was observed among maturity groups after 90 days of storage.



**Fig. 2.** Clove count of the garlic bulb at different times of maturity.

(A) Outer clove count per garlic bulb; (B) Inner clove count per garlic bulb; (C) Total clove count per garlic bulb

Bars with different letters showed a significant difference at 5% level using the LSD test



**Fig. 3.** Secondary metabolites of garlic at different times of maturity stored at varying periods under ambient conditions.

(A) Total phenolic and flavonoid contents and antioxidant activity of garlic at different times of maturity without storage; (B) Comparative total phenolic contents of garlic with different maturity stored at varying storage periods; (C) Quantitative variation in total flavonoid contents of garlic; (D) Comparative analysis of the antioxidant activity in garlic

The antioxidant activity of the different times of maturity was decreased as the storage progressed. However, during storage, the antioxidant activity of 30 and 60 days of storage was similar, but these periods were higher than 90 days of storage. A high antioxidant activity for 100 DAP was observed in 60 days of storage and a decline after this period. But in the case of the 110 DAP maturity group, storage is not needed due to the decline of antioxidant activity. In plants in which bulbs are harvested after 120 DAP, higher antioxidant activity was noted after 30 and 60 days of storage as compared to 90 days of storage. It indicates that every maturity group had a required storage period.

The similarity of the antioxidant activity among the maturity groups during storage, particularly 60 and 90 days of storage, despite there being a slight difference in their TPC and TFC, may be due to the presence of bioactive compounds, such as allicin in garlic powder. The chemical components of garlic are altered during storage (17). But a further study on the allicin and other phenolic compounds is required. The slight change in TPC and TFC during storage may be due to their essential role as antioxidants that scavenge free radicals that can prolong shelf life (18).

## Conclusion

TPC and TFC of unstored garlic powder were not affected by maturity. Likewise, antioxidant activity was higher in maturity groups such as 90 and 110 DAP. In terms of stored garlic powder, the TPC, TFC and antioxidant activity were affected. TPC was

decreased as compared to without storage. Among maturity groups, the TPC in 100 and 120 DAP was higher than that of 90 and 110 DAP. TFC declined with a prolonged storage across maturity groups. TFC of stored garlic powder for 30 to 90 days with a maturity of 90 to 120 DAP was decreased. However, the antioxidant activity was also decreased as the storage progressed. But the highest antioxidant activity for 90, 100, 110 and 120 DAP was observed in 60, 0 and 30 days of storage, respectively. A further analysis of the other secondary metabolites is recommended using the same maturity and storage period.

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## Authors' contributions

FJBD carried out the conceptualization, data curation, methodology, investigation and writing original draft and editing. CBAAB and RJGR carried out the conceptualization, methodology, investigation, data analysis, writing draft and final manuscript. GFSP and MBSGB participated the methodology, data analysis and writing and editing draft and final manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

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