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Proceeding Paper

Nutritional Quality, Bioactive Compounds, and Antioxidant Activity of Nine Clones of Fresh Garlic and Its Black Garlic Derivative: A Comparative Study †

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Abstract: This work focused on the evaluation of nine clones of fresh garlic and its aged product, black garlic, and the comparison of their nutritional qualities, bioactive compounds, antioxidant activities, and the correlation among these traits. The results showed that the moisture content material of black garlic was reduced, while the crude protein, crude fiber, crude ash, and carbohydrate contents were considerably improved. Black garlic had a higher total phenolic content than fresh garlic and four to nine times more antioxidant activity. Hydroxycinnamic acid derivates were the main phenolic acids found in both fresh and black garlic. The antioxidant activity was correlated with polyphenol content and pungency levels. Finally, recommendations about the most suitable clones for black garlic production were made. These findings highlight the need for the consideration of garlic clones in both dietary and therapeutic contexts.

Keywords: *Allium sativum* L.; functional food; biological properties

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1. Introduction

Garlic (*Allium sativum* L.) is one of the main bulbs cultivated and consumed worldwide as a traditional medicinal plant or functional food. White garlic, or fresh garlic, is garlic in its natural state. It has been widely used for cooking because of its pungent flavor and as a medicinal agent because of its biological properties since ancient times. The main bioactive components present in fresh garlic include organosulfur compounds such as alliin, allicin, and different disulfures, and phenol compounds, particularly flavonoids [1]. These bioactive compounds play a role in the reduction in risks of chronic diseases, such as cardiovascular diseases, cancer, and aging-related disorders [2]. A critical drawback of fresh garlic is its strong odor, pungent taste, and the fact that it can cause gastrointestinal discomfort; thus, some people are unwilling to eat fresh garlic. Therefore, among the different commercially available garlic processing products, black garlic, an aged, processed product, has gained importance as an alternative because it has a sweet taste and less pungent odor compared to fresh garlic.

Black garlic is obtained under controlled conditions in temperature and humidity for a certain period of time, which not only modifies the sensory attributes of garlic cloves but also improves their bioactivity [3]. Compared to fresh garlic, black garlic has greater anti-inflammatory and antioxidant effects in vivo and in vitro [4]. The aging process is conducted through a Maillard reaction (non-enzymatic browning), creating the typical dark color of this product [3] and does not require chemical additives or preservatives, keeping its natural character [5].

Previous research has shown that pre-harvest factors, such as genotype and heat treatment conditions, used to produce black garlic are important parameters that affect not only the content of bioactive compounds but also their nutritional quality and biological properties [6–8]. The selection and identification of promising garlic genotypes is one of the main considerations when producing black garlic, because there are significant differences between garlic genotypes in terms of morphological characteristics such as the number, weight, color, and size of cloves and bulbs [9]. Although, the conditions of the aging process determine the changes in color, texture, taste, aroma, moisture content, and the Maillard reaction products that could contribute to the extended shelf-life of black garlic [10].

This work focused on the evaluation of nine clones of fresh garlic and its aged product, black garlic, and the comparison of their nutritional qualities, bioactive compounds (total and individual phenolic compounds), antioxidant activities (by means of the DPPH method), and the correlation among these traits.

2. Material and Methods

2.1. Plant Material

Nine clones of fresh garlic, classified according to ecophysiological groups, were chosen from the germplasm collection of INTA La Consulta: Perla INTA, Ailyn INTA, Nieve INTA, Gran Fuego INTA, Rubí INTA, Morado INTA, Killa INTA, Gigante INTA, and Castaño INTA (Table 1). Black garlic was produced in a ripening chamber for 7 days at 80 °C and 30 days at room temperature.

Table 1. Argentinean classification of garlic clones [11].

Ecophysiological Group	Clones
White type	Perla INTA Ailyn INTA Nieve INTA
Red type	Gran Fuego INTA Rubí INTA
Purple type	Morado INTA Killa INTA
Elephant type	Gigante INTA
Chestnut type	Castaño INTA

2.2. Proximal Composition

The proximal composition was determined by following official analytical methods of the Association of Analytical Communities (AOAC) [12]: for the determination of moisture (AOAC 167.03), dry matter (AOAC 167.03), total minerals (AOAC 942.05), nitrogen (AOAC 984.13), total protein (by calculation: N × 6.25), total fat (AOAC 920.39C), crude fiber (AOAC 962.09), and carbohydrates (by difference).

2.3. Pyruvate Analysis

Pungency levels were analyzed according to González et al. [13]. Garlic cloves were blended for 1 min in distilled water (1:10 *w*/*v*). The juice was collected, filtered, and kept at room temperature for 15 min. A juice aliquot was added to an equal volume of 5% trichloroacetic acid (TCA) and centrifuged for 10 min at 10,000 rpm. One milliliter of 0.0125% 2,4-dinitro-phenylhydrazine (2,4-DNPH) in 2 N HCl was added to 2 mL of diluted juice/TCA (1:20 *v*/*v*). The tubes were incubated at 37 °C for 10 min in a thermostatized bath, and then 5 mL of NaOH 0.6 N was added. The absorbance was measured at 420 nm. The pyruvic acid concentration of the garlic juice was determined using as a reference a standard curve developed with known concentrations of pyruvic acid. Values were expressed as µmol of pyruvic acid per g of fresh weight (µmol pyruvic acid/g fw).

2.4. Total Phenol Content

Total phenol content (TPC) was determined colorimetrically using the Folin–Ciocalteu method, following the procedure previously described by Soto et al. [1]. Briefly, 1 g of each sample was weighed, and 10 mL of methanol (80% v/v) was added. After 1 h of extraction with a magnetic stirrer and 20 min in an ultrasonic bath, the extract was centrifuged at 6000 rpm for 10 min. Each sample was measured against a blank of reagents, containing distilled water instead of extract, after reacting for 10 min. The absorbance was measured at 765 nm. The total phenolic content was determined with a linear calibration curve equation of gallic acid. Values were expressed as mg of gallic acid equivalents per 100 g of dry weight (mg GAE/100 g dw).

2.5. Phenol Profile by UHPLC-DAD Analysis

Chromatographic separation and the simultaneous quantification of phenol profiles were performed according to Lemos et al. [14] using ultra-high-performance liquid chromatography coupled with a diode array detector. The identification of specific compounds was carried out by a comparison of their retention times with those of authentic standards. Standard curves were constructed using commercial reference compounds. Data were expressed as µg per g of fresh weight (µg/g fw). All analyses were conducted in triplicate.

2.6. Antioxidant Activity

Antioxidant activity in vitro was determined by means of the 2, 2-diphenyl-1-picrylhydrazyl radical scavenging method (DPPH), according to Soto et al. [1]. Briefly, 65 µL of the extract (obtained as in Section 2.4) was mixed with 2.5 mL of DPPH solution (40 mg L^{-1} in methanol; absorbance ~ 1.0 at 517 nm). The absorbance at 517 nm was measured after 60 min of incubation at room temperature. Samples were measured (A_E) against methanol and methanol with a DPPH· blank (A_B). The experiment was carried out three times, and the absorbance sample (A_o) was considered. A gallic acid solution (1 mM) was used as a reference (A_{REF}). Radical scavenging activity was calculated in the percentage of inhibition (I%) as follows:

$$\text{Inhibition percentual (I\%)} = [(A_B - (A_E - A_o)/A_B)/(A_B - A_{REF}/A_B)] \times 100$$

2.7. Statistical Analysis

All experimental results were analyzed by an analysis of variance (ANOVA), and a significant difference among means (mean ± SD, $n = 3$) was determined by Tukey's test using InfoStat-Statistical Software2022 (Córdoba, Argentina).

3. Results and Discussion

3.1. Proximal Composition

The proximal composition of fresh and black garlic is presented in Figure 1a–c. Significant differences ($p \leq 0.05$) in the nutritional values were found between clones of the fresh and black garlic evaluated. For fresh garlic, the moisture content varied from 60 g per 100 g (for Castaño INTA) to 68 g per 100 g (for Perla INTA and Killa INTA), while for black garlic, the moisture content ranged to 35 and 59 g per 100 g, for Castaño INTA and Killa INTA, respectively. Taking into account the average of all cultivars from different ecophysiological groups, there were larger differences in moisture content between the chestnut and purple type for fresh garlic and narrower differences between the chestnut and elephant type for black garlic (Table S1). The results showed that after the aging process, the moisture content was reduced by up to 42%. According to similar studies, changes in the moisture content are mainly caused by a reduction in the amount of water from fresh garlic during to the heating process [7,8,15]. For black garlic, the moisture content is a key factor determining its consistency and texture [8].

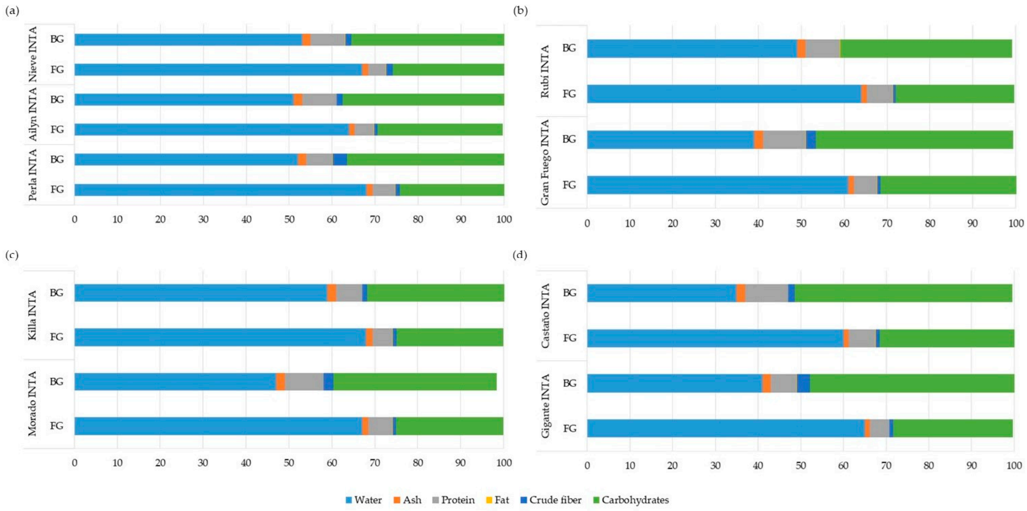


Figure 1. Proximal composition of fresh and black garlic clones classified according to ecophysiological groups: (**a**) white type: Perla INTA, Ailyn INTA and Nieve INTA; (**b**) red type: Gran Fuego INTA and Rubí INTA; (**c**) purple type: Morado INTA and Killa INTA; (**d**) elephant: Gigante INTA and Chestnut type: Castaño INTA. FG: fresh garlic, BG: black garlic.

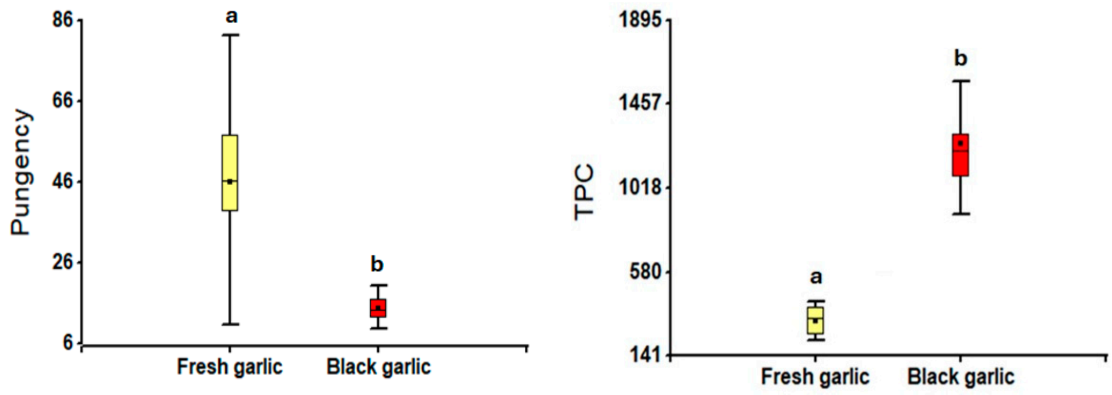
Carbohydrates were the most abundant macronutrients followed by proteins in fresh and black garlic. The highest values of carbohydrates and proteins were found in the clone Castaño INTA. In general, all the black garlic clones showed an increase in protein content, especially those from the red type with an increase of nearly 50%. Our results were similar to those reported by Botas et al. [7] and Tahir et al. [8]. These authors explained that after the aging process, the protein content increased due to enzymatic activity.

With regard to ash content, fresh garlic clones of the purple type showed the highest ash content, while an increasing trend of ash content was observed in all black garlic samples, reaching up to 2 g%g of ash. For fat content, no significant differences were found between fresh and black garlic in all the clones evaluated. The values for crude fiber in fresh garlic differed in a range of 0.6 to 1 g%g for Rubí INTA and Perla INTA, respectively, while for black garlic, the fiber content ranged from 1.1 to 3.4 g%g for Killa INTA and Perla INTA, respectively. The general clones of the red type showed the highest levels of crude fiber.

It was observed that the moisture content of black garlic was reduced, while protein, crude fiber, ash, and carbohydrate contents were considerably increased. This observation was consistent with that reported by Afzaal et al. [16], which stated that aging significantly improved the nutritional composition of black garlic, enhanced the bioavailability of nutrients, and made them easily digested by the body as well.

3.2. Pungency, Total and Individual Phenol Content, and Antioxidant Activity

For pungency levels, there were significant differences between fresh and black garlic ($p \leq 0.05$, Table S2). The average pungency levels expressed as μmol of pyruvic acid per gram of fresh weight were 42.95, 49.48, and 42.95 μmol/g fw for purple, red, and white types of garlic, respectively (Table S3). The lowest levels of pungency were observed in Gigante INTA (elephant type) for fresh garlic and Castaño INTA (chestnut type) for black garlic. In general, fresh garlic cultivars had a 10-fold higher pungency than black garlic clones ($p \leq 0.05$, Figure 2a). Rubí INTA was the fresh garlic clone with the highest pungency, while among the black garlic clones, Morado INTA showed the highest levels of pungency (Table S2).



(**a**) (**b**)

Figure 2. Comparative boxplot of: (**a**) pungency levels (μmol pyruvic acid/g fw) and (**b**) total phenolic content (TPC, mg EAG/100 g dw) of fresh and black garlic. Different superscript letters indicate a significant difference ($p \leq 0.05$).

The total polyphenol content is shown in Tables S2 and S3, while comparative levels between fresh and black garlic are presented in Figure 2b. Significant differences among all the fresh and black garlic clones ($p \leq 0.05$) were found for the total polyphenol content. The highest concentration of total polyphenol content was observed in the chestnut type and the lowest concentration in the red type. Among individual clones, for fresh garlic, Perla INTA showed the highest total phenol content and Rubí INTA the lowest content, while for black garlic, Morado INTA showed the highest concentration and Castaño INTA the lowest concentration. It was found that black garlic presented a higher total phenolic content (1028.84–1727.95 mg EAG/100 g dw) than fresh garlic (228.49–403.65 mg EAG/100 g dw), and hydroxycinnamic acid derivates were found to be the main phenolic acids in both fresh and black garlic (Figure 2b and Tables S2–S7). A typical chromatogram is shown in Figure S1. All black garlic samples showed an increase in the phenolic compounds between 3 and 6 times compared to fresh garlic samples. Previous studies have described an increase of 2–3 times in polyphenol content compared to raw garlic [10,17,18]. This increase in the polyphenol content in black garlic is concordant in part to results reported by other authors, who found a threefold increase in content [17].

The antioxidant activity estimated by the DPPH method revealed significant and wide variation in fresh garlic clones, in a range of 1% to 13%, while it in black garlic clones increased from 60% to 98% ($p \leq 0.05$, Table S2). With regard to the ecophysiological group, the red type and the chestnut type showed the highest antioxidant activities for fresh and black garlic, respectively, while the elephant type had the lowest antioxidant activity for both fresh and black garlic. Perla INTA and Morado INTA had the highest antioxidant activities for fresh and black garlic, respectively, while Gran Fuego INTA and Gigante INTA showed the lowest antioxidant activities among all fresh garlic samples. Gigante INTA showed the lowest antioxidant activity among the black garlic samples. It was found that black garlic had four to nine times more antioxidant activity than fresh garlic (Figure 3). Previous studies have described that black garlic has a higher antioxidant activity than fresh garlic in both in vitro and in vivo assays [10,18]. Our results were in agreement with previous reports which reported that antioxidant activity increases in aged black garlic, reaching 25-fold compared to that of fresh garlic [8,19–22].

A pairwise correlation analysis among all the variables revealed a positive and strong correlation between the total polyphenol content and pungency levels with antioxidant activity ($p \leq 0.001$, Table 2). Our results indicated that the main contributors to antioxidant activity in fresh and black garlic were TPC and organosulphur compounds (expressed in pungency levels).

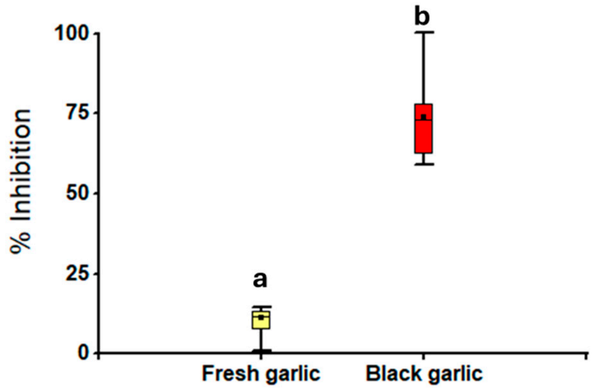


Figure 3. Comparative boxplot of DPPH scavenging activity of fresh and black garlic. Different superscript letters indicate a significant difference ($p < 0.05$).

Table 2. Pearson correlation coefficient ® between total phenolic content, pungency, and antioxidant activity.

	Pungency	TPC	DPPH (I%)
Pungency	-	−0.66	0.59
TPC [1]	−0.66	-	0.97
DPPH [2] (I%)	0.59	0.97	-

[1] TPC: total phenol content; [2] DPPH (I%): antioxidant activity.

4. Conclusions

This comparative study provides information on the nutritional quality, bioactive compounds, and antioxidant activity of fresh and black garlic clones. The results provided evidence that there is great variability among garlic clones that belong to different ecophysiological groups and also among clones that belong to the same ecophysiological group for all the analyzed variables. Fresh garlic from the purple and the chestnut types, due to their high content of crude fiber, protein and carbohydrates, and high levels of bioactive compounds, emerged as the superior candidates for black garlic production. With regard to individual clones, the black garlic types Morado INTA and Castaño INTA could be considered as functional food, as they showed the highest levels of phenol content and antioxidant activity. Moreover, the strong correlations found suggested that the garlic antioxidant activity is mainly due to the organosulfur compounds (expressed as pungency levels) and phenol content. Furthermore, this study could allow us to choose the fresh garlic clones that could be suitable to obtain the black garlic with the best nutritional characteristics. These findings highlight the need for a consideration of garlic clones in both dietary and therapeutic contexts.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/blsf2024040029/s1, Table S1. Proximal composition of fresh and black garlic clones classified by ecophysiological group; Table S2. Pungency levels, total phenol content, and antioxidant activity of fresh and black garlic clones; Table S3. Pungency levels, total phenol content, and antioxidant activity of fresh and black garlic classified by ecophysiological group; Table S4. The phenolic acid constituents of white garlic type; Table S5. The phenolic acid constituents of red garlic type; Table S5. The phenolic acid constituents of purple garlic type; Table S7. The phenolic acid constituents of elephant and chestnut garlic types. Figure S1. Chromatograms corresponding to phenolic compounds analyzed.

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