

# Colorimetric, chemical, and genetic characterization of purple garlic in two producing regions in Brazil











**Abstract** – The objective of this work was to compare the purple garlic cultivated in the Planalto Catarinense region with the one in Midwestern/Southeastern region of Brazil, concerning colorimetric and chemical aspects, as well as genetic patterns. The color of the bulbils was determined through colorimetric analysis, the volatile compounds were characterized by solid phase micro-extraction and gas chromatography, and the genetic diversity was analyzed using microsatellite markers. Garlic bulbs grown in the Planalto Catarinense region have a more intense purplish color. The samples from the Midwestern/Southeastern region present a significantly lower concentration of volatile compounds. There is no consistent genetic difference between samples from the Planalto Catarinense and Midwestern/Southeastern regions. The purple garlic from each cultivation region has singularities, mainly due to the farmers' management method and edaphoclimatic factors. The uniqueness presented through the colorimetric and chemical analyses fulfills the basic requirements that are necessary for the Geographical Indication of these products.

**Index terms:** *Allium sativum*, geographical variation, genetic diversity, microsatellite markers.

## Caracterização colorimétrica, química e genética de alho-roxo em duas regiões produtoras do Brasil

**Resumo** – O objetivo deste trabalho foi comparar o alho-roxo cultivado na região do Planalto Catarinense com o cultivado na região Centro-Oeste/Sudeste do Brasil, com relação aos aspectos colorimétricos e químicos, assim como aos padrões genéticos. A coloração dos bulbilhos foi determinada por análise colorimétrica, a caracterização dos compostos voláteis foi conduzida por microextração em fase sólida e cromatografia gasosa, e a diversidade genética foi analisada com marcadores microsatélite. Os bulbos de alho cultivados no Planalto Catarinense têm uma coloração mais intensa. As amostras da região Centro-Oeste/Sudeste apresentam uma concentração significativamente menor de compostos voláteis. Não há diferença genética consistente entre as amostras do Planalto Catarinense e das regiões Centro-Oeste/Sudeste. O alho-roxo de cada região de cultivo tem singularidades, principalmente em razão do método de manejo do produtor e de fatores edafoclimáticos. A singularidade apresentada nas análises colorimétricas e químicas atende aos requisitos básicos necessários para a Indicação Geográfica destes produtos.

**Termos para indexação:** *Allium sativum*, variação geográfica, diversidade genética, marcadores microsatélite.

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## Introduction

Garlic (*Allium sativum* L.), which is native to Asia, has conquered tastes worldwide due to its distinctive fragrance and flavor, becoming a significant presence in global cuisine. Garlic bulbs play a crucial role as an agricultural crop, having China as the main producing country, leading the cultivation on a global scale (Sui et al., 2022).

Garlic is a functional food because it contains several phytochemical elements with significant biological activities, including sulfur compounds, pectin, fructans, carbohydrates, essential amino acids, and proteins (Kovarovič et al., 2019; Ezeorba et al., 2022). The key sulfur-containing amino acid in garlic is alliin, specifically S-allyl-L-cysteine sulfoxide, representing 1.29% of fresh garlic content (Kovarovič et al., 2019). The amount of this compound, which is responsible for the main sensorial characteristics, is influenced by environmental factors, such as climate and soil (Kovarovič et al., 2019). Alliin is susceptible to degradation, especially at high temperatures, due to unstable sulfoxide bonds (Chen et al., 2017; Kovarovič et al., 2019).

In general, the characteristics of an agricultural product are the result of a set of factors, such as soil and climate conditions, management, selection, and genetic patterns. These factors give certain products unique characteristics compared with their equivalents from different locations or under diverse conditions. Such singularities have been the ground for Geographic Identity certifications. In turn, terroir is an important alternative for sustainable territorial development due to the uniqueness and consequent valorization of the agricultural products.

The Planalto Catarinense region, located in the state of Santa Catarina, is a reference in the production of purple garlic in the South of Brazil, being produced mainly by family farming on small properties (Landau et al., 2020). The region naturally meets the needs concerning the eco-physiological conditions for purple garlic cultivation: more than 13 hours of light per day and low temperatures that favor bulb formation (Resende et al., 2024). Additionally, the product has been improved through the selection and propagation of plants that preserve characteristics such as color and size (Gugel, 2024).

Despite the edaphoclimatic needs for garlic cultivation, vernalization technologies allowed the

establishment of garlic farms in regions with adverse conditions (Lopes et al., 2016), such as the Midwestern and Southeastern regions of Brazil. In spite of being dependent on pre-planting vernalization and requiring artificial control of several factors, such as temperature, light time, and humidity to obtain an adequate result (Resende et al., 2020), the garlic production in these areas has been growing significantly. For instance, in 2022, the state of Goiás, in the Midwestern region, produced 58,459 t of garlic in an area of 3,440 ha, while Planalto Catarinense and other small regions in the state of Santa Catarina produced 14,365 t in an area of 1,580 ha (IBGE, 2024).

The objective of this work was to compare the purple garlic cultivated in the Planalto Catarinense region with the one in the Midwestern/Southeastern region of Brazil, concerning colorimetric, chemical, and genetic patterns.

## Materials and Methods

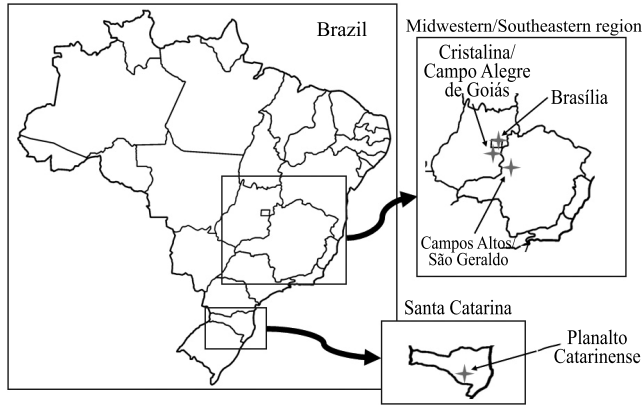
The climate of the Planalto Catarinense region, according to the Köppen-Geiger classification, is type Cfb, temperate, without dry season, and cool summer, with an average precipitation of 1,669.7 mm annually, and an average temperature of 16.5°C (Climate Data, 2024).

The municipalities of the Midwestern and Southeastern regions preset different climates. The climate in Cristalina, Campo Alegre de Goiás, and Brasília is classified as AW, tropical savannah climate, with a drier season in winter, with an annual rainfall of 1,454.7 mm and an average temperature of 22°C (Climate Data, 2024). São Gotardo climate is of Cwa type, humid subtropical climate, with a hot summer, with an annual rainfall of 1,570 mm and an average temperature of 20.5°C, while the climate of Campos Altos is classified as Cwb, altitude subtropical climate, with a cool summer, presenting an annual precipitation of 1,552 mm and average temperature of 20.3°C (Climate Data, 2024).

Considering that the seed bulbs used in both regions have the same origin, the hypothesis was that due to edaphoclimatic conditions of the cultivation areas, there are reliable differences in the color and chemical composition of purple garlic produced in each region, without significative genetic discrepancies.

The sampling in the Planalto Catarinense region (Figure 1) was carried out in the second half of

November 2021. Plants were selected randomly, in the field or in the shed of each farmer, totaling 17 collection points: IG21A01 to IG21A17 (Table 1). Garlic samples from the Midwestern and Southeastern



**Figure 1.** Map of garlic collection points in the Planalto Catarinense and Midwestern/Southeastern regions, Brazil. Stars represent the approximate location of the municipalities where garlic samples were cultivated.

regions (Figure 1) were collected in the same period, directly from farmers or in markets in the municipality of Florianópolis, state of Santa Catarina, totaling six samples: IG21B01 to IG21B06 (Table 1).

To conduct colorimetric analysis, the leaves and roots of each sample were removed, and the tunic was detached carefully to preserve the bulbils. The color of the bulbils was determined using a CR400 colorimeter (Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA) in the form of lightness ( $L^*$ ), bluish-green/red-purple hue ( $a^*$ ), and yellow/blue hue ( $b^*$ ) color spaces. Five random bulbils from each sample were evaluated, with readings in the central part of the three sides of each bulb. The hue angle was calculated as  $h^\circ = (\arctangent(b^*/a^*) / 2\pi) \times 360$  and the chroma angle as  $C^* = (a^{*2} + b^{*2})^{1/2}$  (McGuire, 1992). The difference between regions concerning the hue angle estimations was determined using the Mann-Whitney's test, at 5% significance, computed using the R package version 4.3.2 (R Core Team, 2019).

The volatile components of the garlic samples were extracted by solid-phase microextraction, being

**Table 1.** Luminosity ( $L^*$ ) measures for garlic samples from the Planalto Catarinense and Midwestern/Southeastern regions by municipality. Values are the median over repetitions.

| Region                  | Sample                 | Cultivar      | Municipality              | $L^*$ |
|-------------------------|------------------------|---------------|---------------------------|-------|
| Planalto Catarinense    | IG21A01                | Chonan        | Lebon Régis, SC           | 47.70 |
|                         | IG21A02                | Chonan        | Caçador, SC               | 53.77 |
|                         | IG21A03                | Chonan        | Curitibanos, SC           | 45.81 |
|                         | IG21A04                | Chonan        | Frei Rogério, SC          | 46.30 |
|                         | IG21A05                | Chonan        | Fraiburgo, SC             | 53.15 |
|                         | IG21A06                | Chonan        | Caçador, SC               | 49.59 |
|                         | IG21A07                | Chonan        | Lebon Régis, SC           | 49.23 |
|                         | IG21A08                | Chonan LV GR5 | Frei Rogério, SC          | 47.10 |
|                         | IG21A09                | Chonan        | Fraiburgo, SC             | 48.03 |
|                         | IG21A10                | Ito           | Caçador, SC               | 50.23 |
|                         | IG21A11                | Ito           | Frei Rogério, SC          | 45.45 |
|                         | IG21A12                | Ito           | Frei Rogério, SC          | 51.43 |
|                         | IG21A13 <sup>(1)</sup> | Ito           | Fraiburgo, SC             | 51.59 |
|                         | IG21A14                | Ito           | Fraiburgo, SC             | 48.17 |
|                         | IG21A15                | Jonas         | Curitibanos, SC           | 43.45 |
|                         | IG21A16                | Contestado    | Frei Rogério, SC          | 44.87 |
|                         | IG21A17                | Caçador 40    | Campo Belo do Sul, SC     | 47.70 |
| Midwestern/Southeastern | IG21B01                | Quitéria      | Cristalina, GO            | 61.61 |
|                         | IG21B02                | Ito           | Brasília, DF              | 59.19 |
|                         | IG21B03                | São Valentim  | Campo Alegre de Goiás, GO | 59.84 |
|                         | IG21B04                | Ito           | Campo Alegre de Goiás, GO | 57.25 |
|                         | IG21B05                | Undefined     | Campos Altos, MG          | 59.71 |
|                         | IG21B06                | Undefined     | São Gotardo, MG           | 53.96 |

<sup>(1)</sup>Sample with evidence of bacterial disease. SC, Santa Catarina state; GO, Goiás state; and MG, Minas Gerais state.

separated and identified using gas chromatography coupled with mass spectrometry (Ropelewska et al., 2022). Each sample was analyzed twice. For the volatile compound extraction, 100 µm PDMS fiber and 40 mL bottles with lids containing a silicone septum were used. All samples were processed and frozen in vials before analysis. Bottles were prepared with  $1.0 \pm 0.002$  g of ground garlic, in triplicate. For extraction, the vials with the samples were added to a magnetic stirrer with 15 mL of saturated aqueous NaCl solution and 50 µL of 4 methyl-2-pentanol solution at 2 µL mL<sup>-1</sup> in ethanol as internal standard. The vials were transferred to a water bath at 45°C for 5 min and the fiber was exposed in the headspace for 30 min at 45°C to adsorb the volatile components.

The fiber was transferred to the injector of a gas chromatograph coupled to a 7890A mass spectrometer (Agilent, Santa Clara, CA, USA), operating at 250°C. Chromatographic separation was performed on an Agilent HP-5ms column (5% phenyl-polydimethylsiloxane; 30 cm x 250 µm x 0.25 µm), using a temperature ramp from 40 (5 min) to 90°C at a rate of 5°C min<sup>-1</sup>, followed by a temperature rise to 250°C at a rate of 12°C min<sup>-1</sup> and a waiting time of 2 min (Biancolillo et al., 2022). The interface temperature between the gas chromatograph and mass spectrometer was set to 300°C. Helium gas was used as carrier gas at a flow rate of 1 mL min<sup>-1</sup> and the mass detector was operated by 70 eV electron impact in a range of 35–450 mHz.

Relative percentages of the compounds were obtained from the average of six analyses using the G1701EA GC/MSD Chemstation software. The substances present in the oil were characterized by comparing the mass spectrum and experimental Kovats index (KI) for each component with the respective mass spectra and Kovats index of standards (Adams, 2007). The experimental KI values were obtained from the injection of a sample of C7-C30 saturated hydrocarbons (Merck KGaA, Darmstadt, Germany) under the same conditions.

For molecular genetic analyses, total DNA was isolated from approximately 100 mg of bulbil tissue from each sample, using the CTAB protocol (Doyle & Doyle, 1990). The quantity and quality of the isolated DNA was verified using a NanoDrop 1000 version 3.8 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and horizontal electrophoresis in

0.8% agarose gel, stained with Gelred, and visualized using a UV light transilluminator.

Thirteen SSR markers, developed for *Allium sativum* were employed: ASA08, ASA10, ASA16, ASA17, ASA24, ASA25, ASA31 (Cunha et al., 2012), GB-ASM40, GB-ASM53, GB-ASM59, GB-ASM72, GB-ASM78, and GB-ASM80 (Ma et al., 2009). The markers were selected based on their reported polymorphism and ability to distinguish the studied garlic varieties.

The microsatellite markers were amplified via PCR reaction in a Veriti thermal cycler (Thermo Fisher Scientific, Waltham, MA, USA), using the M-13 tail methodology with fluorescent labeling (Schuelke, 2000). The PCR mix contained 50 ng DNA, 2.5 mM MgCl<sub>2</sub>, 0.2 µM Primer Forward, 0.8 µM Primer Reverse, 0.8 µM FAM Fluorescently Labeled Primer M-13 (5'-FAMTGTAACGACGGCCAGT-3'), 0.2 mg mL<sup>-1</sup> BSA, 0.2 µM of each dNTP, 1X PCR buffer, and 1U Taq DNA polymerase, in a volume of 15 µL.

The amplification cycle consisted of 3 min at 94°C, followed by 30 cycles of 40 seconds at 94°C, 45 s at the annealing temperature of each primer pair, 45 s at 72°C, and eight cycles of 30 s at 94°C, 45 s at 53°C, and 45 s at 72°C, ending with an extension step of 10 min at 72°C. In this methodology, all forward primers have the M-13 sequence (5'-TGTAACGACGGCCAGT-3') at its 5' portion.

After amplification, the SSR alleles were resolved by capillary electrophoresis in an ABI 3500xL Genetic Analyzer. The identification of the alleles was carried out using the GeneMapper software for automatic genotyping. A manual check on the recorded peaks was performed to correct any genotyping error.

Data analysis was performed using the programs GenAlEx v. 6.5 (Peakall & Smouse, 2012) and Fstat 2.9.4 (Goudet, 2003). The data were structured into two groups, one containing the samples from the Planalto Catarinense region (PC group) and the other containing the samples from the Midwestern and Southeastern regions (MW/S group). Initially, a probability of identity (PI) analysis was performed to evaluate the informative capacity of the SSR markers employed. This analysis calculates the likelihood of two randomly chosen samples having the same genotype for the used markers, considering the subsequent addition of new loci to the analysis.



The total number of alleles ( $A$ ), the effective number of alleles ( $A_e$ ), the allelic richness ( $A_R$ ), the Shannon entropy index ( $I$ ), the unbiased expected heterozygosity ( $H_e$ ), the observed heterozygosity ( $H_o$ ), and the fixation index ( $F$ ) were estimated for each locus individually and for all loci. The partition of differentiation between groups was estimated based on Shannon's entropy index. A principal coordinate analysis (PCoA) based on the genetic distance between pairs of samples was performed to graphically represent their relationship.

A Bayesian model-based clustering analysis (Pritchard et al., 2000) was implemented using the non-admixture and the frequency-independent alleles models with 100,000 Markov Chain Monte Carlo steps and 50,000 burn-in periods using the software STRUCTURE version 2.3.4 (Pritchard et al., 2000). The number of  $K$  was set from 1 to 10 and ten replicates were run for each  $K$ . The optimum number of clusters  $K$  was selected using the approach suggested by Evanno et al. (2005), as implemented in the Python module of the Structure Harvester software (Earl et al., 2012).

## Results and Discussion

For the luminosity ( $L^*$ ) data (Table 1), there was a trend indicating that the samples from the PC group (Figure 2 A) have more intense coloration of the bulbs, compared to the samples of the MW/S group (Figure 2 B). An even more evident trend was observed at the correlation between the chroma values ( $C^*$ ) and hue values ( $h^\circ$ ) (Figure 2 C): all samples have similar  $C^*$ , representing the purity or intensity of a color in the red-purple color region, according to the chromaticity diagram with  $h^\circ$  (Table 2), varying between  $12.84^\circ$  (IG21A16) and  $57.60^\circ$  (IG21B05). However, the samples from the PC group presented lower values ( $W = 120$ ;  $p\text{-value} = 0.00098$ ) for  $h^\circ$  (Table 2 and blue area of Figure 2 C) compared to samples from the MW/S group (Table 2 and red area of Figure 2 C), indicating that the color of the garlic produced in the Planalto Catarinense region is closer to purple than the garlic from Midwestern and Southeastern regions.

The differentiation between the samples is mainly due to the values corresponding to the hue angle (Table 2). While the  $C^*$  values (grayish area of Figure 2 C) overlap for the samples from the PC and the MW/S groups, there is no overlap between the

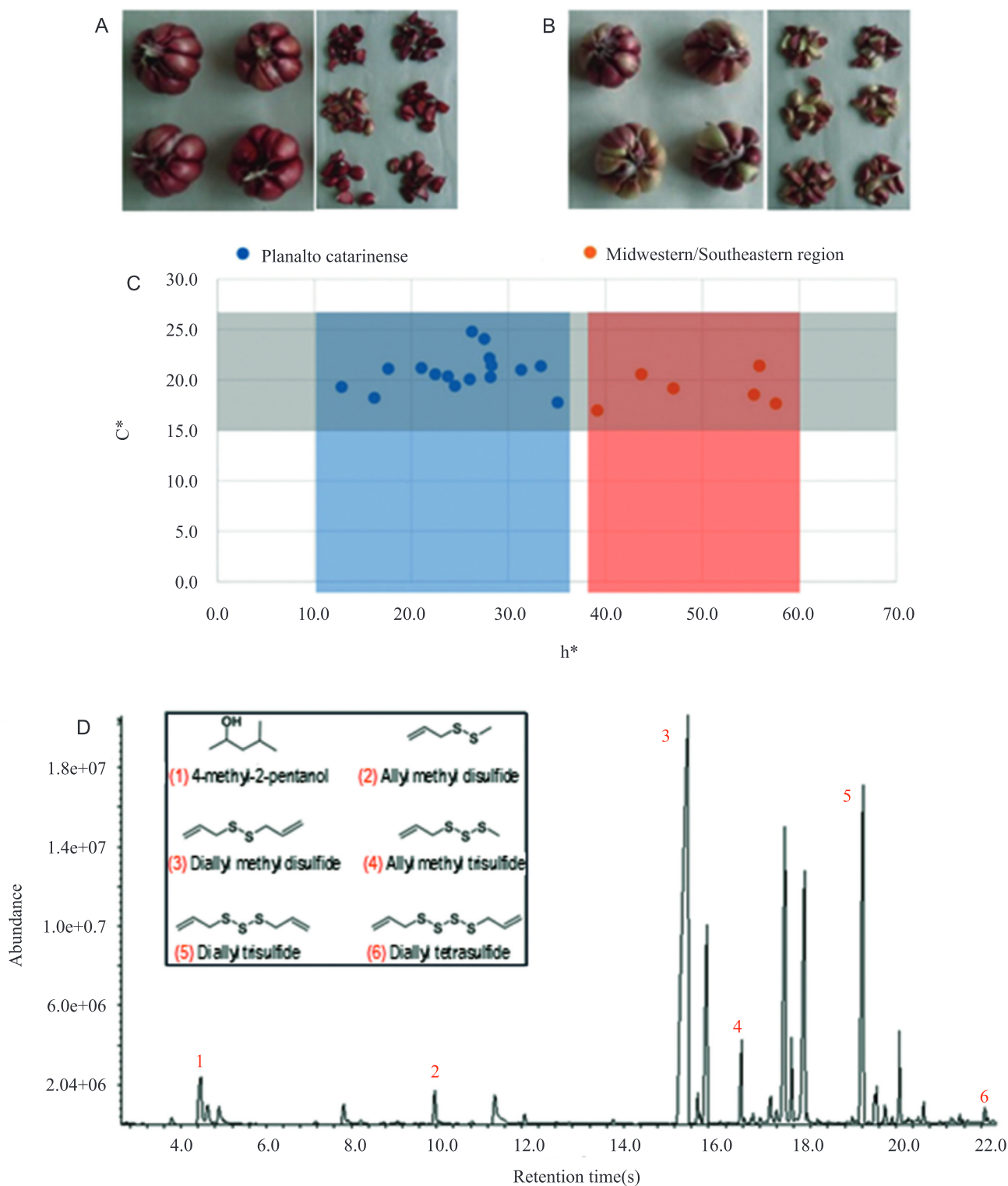
samples for the  $h^\circ$  values (blue area between 10 and 35 for the samples of PC group and red area between 39 and 60 for the samples of MW/S group in Figure 2 C).

All samples, from both PC and MW/S groups, showed the same chemical composition concerning the volatile components from the allicin degradation: allyl methyl disulfide, diallyl methyl disulfide, allyl methyl trisulfide, diallyl trisulfide, and diallyl tetrasulfide (Figure 2 D). However, significant differences ( $t\text{-test} = 2.48273$ ,  $p = 0.021$ ) were observed regarding the total area of these five volatile components from each sample in each region. The summation of the individual area of each peak was 25.447 (SD = 5.27) for the PC group and 19.72 (SD = 3.09) for the MW/S group, revealing the production of volatile compounds 1.29-fold higher in the garlic cultivated in the Planalto Catarinense region.

Components derived from allyl thiosulfonates are rapidly formed when the bulbs undergo cell rupture or when the dry or pulverized material is moistened (Lawson & Hunsaker, 2018). These allyl thiosulfonates, or their degradation products (allyl polysulfides) and other common metabolites, such as allyl methylsulfide, have been considered responsible for most of the pharmacological activities found in garlic. Allicin is the main component among allyl thiosulfonate derivatives and can consist of up to 91% of the mixture components (Lawson & Hunsaker, 2018). However, allicin and other allyl thiosulfonates are unstable and are spontaneously degraded to turn into allyl polysulfide derivatives.

The probability of identity (PI) assessment (Figure 3 A) demonstrated that the chance of two randomly selected samples within each group having the same genotype for the 13 loci only by chance is  $PI_{PC} = 0.000023$  for the samples from the PC group and  $PI_{MW/S} = 0.000021$  for the samples of the MW/S group. This result confirms the high informative capacity of the markers.

The estimated genetic diversity indexes demonstrate that samples from the two regions have remarkably similar characteristics. The multilocus mean estimates for the PC and the MW/S groups presented almost identical results for all measured indexes (Table 3). The effective number of alleles ( $A_e = 1.18$ ) is approximately 50% of the total number of alleles ( $A = 2.15$  and  $A = 2.08$ ), indicating that some alleles have relatively low frequency. Such a pattern deserves



**Figure 2.** Colorimetric analysis and chemical pattern of volatile compounds of garlic. Bulbs and bulbils of 'Ito' (IG21A11) grown in the Planalto Catarinense region (A). Bulbs and bulbils of 'Ito' (IG21B02) grown in the Midwestern region (B). Graphic of chroma ( $C^*$ ) versus hue angle ( $h^\circ$ ) for garlic from the Planalto Catarinense (blue dots) and Midwestern/Southeastern (red dots) regions (C). Gas chromatogram of a garlic sample: peak 1 (4-methyl-2-pentanol) is the internal standard of the analysis, while peaks 2 to 6 are the volatile products of the alliin degradation (D). Photos by Volni Mazzuco.

attention since it may result in the loss of low-frequency alleles in further generations. On the other hand, the higher estimations of observed heterozygosity compared with the expected heterozygosity may be caused by the selection of bulbs to establish new garlic fields. This feature also suggests the presence of high genetic diversity, despite the predominantly clonal propagation of the species.

The estimated differentiation between the PC and MW/S groups was 1% using the Shannon entropy index (Figure 3 B). This result suggests no significant genetic differentiation between the samples cultivated in the Planalto Catarinense and the Midwestern/Southeastern regions. Thus, the genetic differentiation

in the samples is predominantly among individuals/varieties (Figure 3 B).

The principal coordinate analysis (PCoA) based on the genetic distance between pairs of samples calculated from the genotypic data of the 13 SSR markers (Figure 3 C) reflects the data estimated in the other analyses, demonstrating high genetic identity between the samples from different regions, without grouping them according to their geographic origin. The analysis represented 59.98% of the total variation of the data set in its first two axes.

The same conclusion concerning high similarity between samples of both regions could be observed using the model-based Bayesian analysis. None of

**Table 2.** Measurements of the color space components  $a^*$  and  $b^*$ , hue angle ( $h^\circ$ ), and chroma ( $C^*$ ) for garlic samples from the Planalto Catarinense and Midwestern/Southeastern regions. Values are the median over repetitions.

| Region                  | Sample                 | $a^*$ | $b^*$ | $h^\circ$ | $C^*$ |
|-------------------------|------------------------|-------|-------|-----------|-------|
| Planalto Catarinense    | IG21A01                | 18.27 | 6.75  | 20.28     | 19.48 |
|                         | IG21A02                | 15.94 | 10.43 | 33.20     | 18.88 |
|                         | IG21A03                | 17.75 | 6.94  | 20.64     | 18.74 |
|                         | IG21A04                | 18.74 | 6.74  | 19.62     | 19.92 |
|                         | IG21A05                | 15.21 | 9.16  | 31.03     | 17.75 |
|                         | IG21A06                | 17.01 | 7.45  | 22.99     | 18.40 |
|                         | IG21A07                | 15.98 | 7.86  | 26.19     | 17.81 |
|                         | IG21A08                | 17.52 | 6.72  | 20.36     | 18.76 |
|                         | IG21A09                | 19.19 | 9.07  | 25.31     | 21.09 |
|                         | IG21A10                | 19.45 | 11.85 | 31.01     | 23.04 |
|                         | IG21A11                | 21.28 | 10.89 | 27.10     | 23.95 |
|                         | IG21A12                | 14.15 | 7.79  | 28.83     | 16.31 |
|                         | IG21A13 <sup>(1)</sup> | 16.07 | 11.46 | 36.09     | 19.97 |
|                         | IG21A14                | 19.93 | 10.72 | 28.02     | 22.42 |
|                         | IG21A15                | 18.70 | 5.42  | 16.16     | 19.47 |
|                         | IG21A16                | 19.82 | 9.11  | 24.69     | 21.81 |
|                         | IG21A17                | 18.27 | 6.75  | 20.28     | 19.48 |
| Midwestern/Southeastern | IG21B01                | 11.72 | 13.68 | 49.43     | 18.02 |
|                         | IG21B02                | 15.17 | 15.18 | 45.02     | 21.46 |
|                         | IG21B03                | 14.68 | 14.17 | 43.91     | 20.37 |
|                         | IG21B04                | 13.48 | 14.77 | 47.62     | 20.24 |
|                         | IG21B05                | 10.31 | 16.59 | 58.14     | 19.46 |
|                         | IG21B06                | 11.18 | 14.91 | 53.19     | 18.66 |

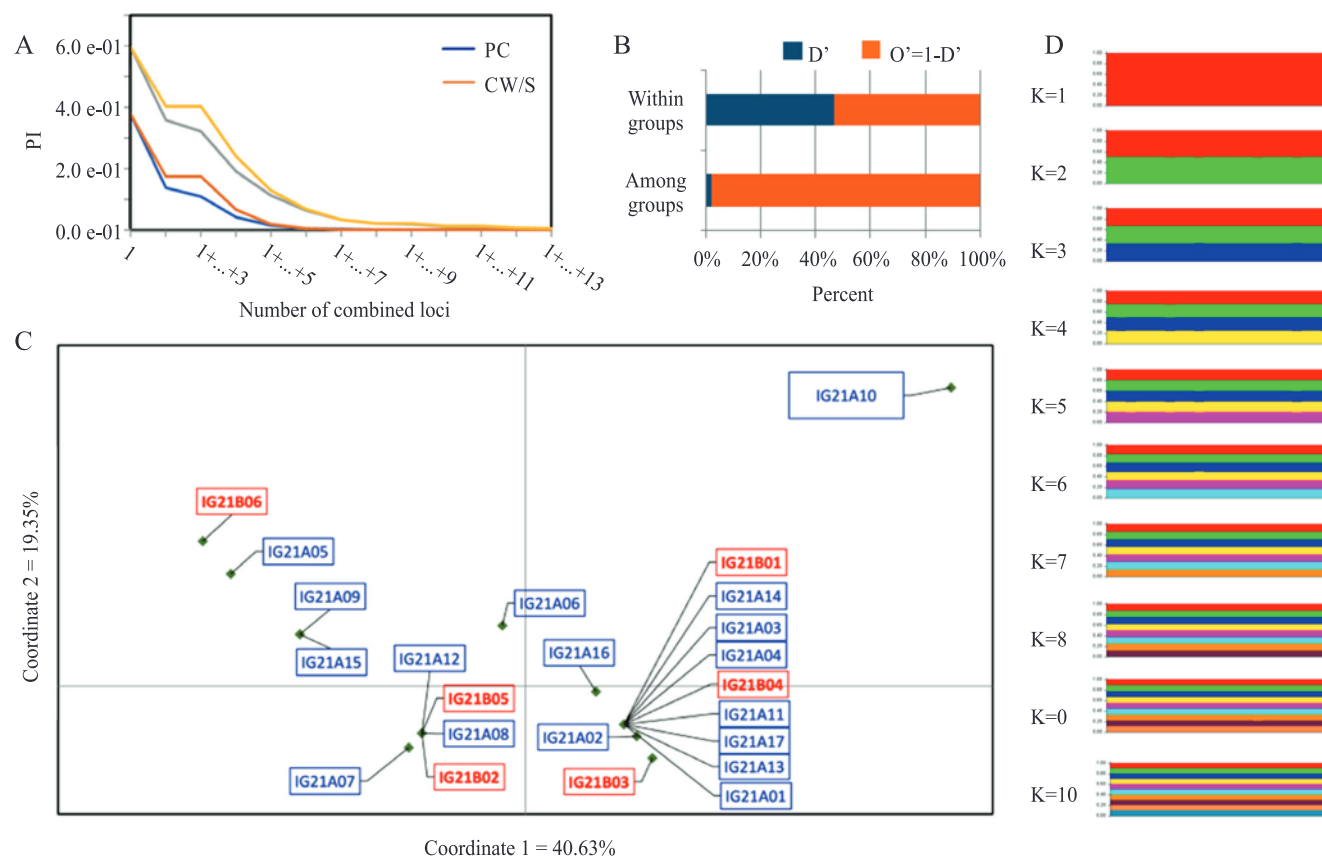
<sup>(1)</sup>Sample with evidence of bacterial disease. The corresponding cultivar and municipality of cultivation are the same as reported in Table 1.

the determined clusters (K) revealed differentiation between the regions. All samples have the same membership in all clusters despite the number of K (Figure 3 D).

In general, molecular genetic analyses using microsatellite markers did not reveal significant differences among samples from Planalto Catarinense and Midwestern/Southeastern regions; however, it is crucial to highlight that the number of samples was restricted. Therefore, the low genetic divergence may be associated with the fact that the garlic seed used in the Midwestern/Southeastern region often comes from the Planalto Catarinense region, this is, they present the same genetic background.

Other studies aimed at the genetic characterization of garlic, for instance, some reported the formation of groups related to photoperiod requirements and growth cycles (Buso et al., 2008; Cunha et al., 2014), as well as the time of maturity and flowering (Mota et al., 2004; Volk et al., 2004; Panthee et al., 2006), but there are no known works on the geographical origin area of the samples.

Morphological and genetic singularities have been described and used in the Denomination of Origin model to valorize and protect some agricultural products through Geographical Indication (Brasil, 2023). In Santa Catarina state, such genetic characterization was performed, for instance, for the characterization of evolutive molecular traits of



**Figure 3.** Genetic analyses of garlic samples from Planalto Catarinense (PC) and Midwestern/Southeastern (MW/S) groups. Probability of identity of the SSR markers used in the genetic evaluation (A). Partition of diversity within and between PC and MW/S groups, based on the Shannon entropy index, where D' is the diversity component and de O' is the overlapping component (B). Principal coordinate analysis (PCoA) of garlic samples from PC (blue) and MC/S (red) groups based on genetic distance among samples (C). Model-based Bayesian clustering analysis for one to ten clusters K (represented by different colors) for the 23 samples (D). The membership of all samples is quite similar in all values of K.



**Table 3.** Mean estimates and standard error of the total number of alleles (A), effective number of alleles (Ae), allelic richness (A<sub>R</sub>), Shannon's entropy index (I), observed heterozygosity (H<sub>o</sub>), expected heterozygosity (H<sub>e</sub>), and fixation index (F) overall 13 loci, for the Planalto Catarinense (PC) and Midwestern/Southeastern (MWS) regions and overall samples.

| Allele and index | PC           | CW/S         | Overall      |
|------------------|--------------|--------------|--------------|
| A                | 2.15 (0.15)  | 2.08 (0.24)  | 2.11 (0.14)  |
| Ae               | 1.18 (0.12)  | 1.18 (0.15)  | 1.18 (0.09)  |
| A <sub>R</sub>   | 1.90 (0.32)  | 1.97 (0.49)  | 1.91 (0.35)  |
| I                | 0.59 (0.08)  | 0.59 (0.10)  | 0.59 (0.06)  |
| H <sub>o</sub>   | 0.74 (0.12)  | 0.72 (0.12)  | 0.73 (0.08)  |
| H <sub>e</sub>   | 0.42 (0.06)  | 0.43 (0.07)  | 0.42 (0.05)  |
| F                | -0.65 (0.17) | -0.72 (0.06) | -0.72 (0.09) |

the Pacific oyster cultivated in Santa Catarina Island (Stefenon & Clauman, 2024) and for the yeasts used in the fermentation of the artisanal cachaça and brandy produced in Luis Alves (Stefenon et al., 2021; INPI, 2024). Both studies demonstrated the genetic singularities of the products, aiming at the Geographical Indication.

## Conclusions

1. Garlic bulbs grown in the Planalto Catarinense region have a more intense purplish color.
2. The samples from the Midwestern/Southeastern region present a significantly lower concentration of volatile compounds.
3. There is no consistent genetic difference between samples from the Planalto Catarinense and Midwestern/Southeastern regions.
4. The Purple garlic from each cultivation region has singularities, mainly due to the farmers' management method and the edaphoclimatic factors.
5. The uniqueness presented through the colorimetric and chemical analyses fulfills the basic requirements that are necessary for the Geographical Indication of these products.

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