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Original Article

Subzero vernalization improves post-harvest quality of noble garlic grown in semi-arid region[1]

Vernalização com temperaturas negativas melhora a qualidade do alho nobre cultivado no semiárido

Éric G. Morais[2*], Leilson C. Grangeiro[2], Dalbert de F. Pereira[2], Renata R. T. Oliveira[2], Elidayane da N. Santos[2], Ilmara B. M. Silva[2], Lucas M. da S. Sousa[2], Romualdo M. C. Costa[2], Bruna de P. Souza[2] & Elizangela C. dos Santos[2]

[1] Research developed at Universidade Federal Rural do Semi-Árido, Departamento de Ciências Agronômicas e Florestais, Mossoró, RN, Brazil
[2] Universidade Federal Rural do Semi-Árido/Departamento de Ciências Agronômicas e Florestais, Mossoró, RN, Brazil

HIGHLIGHTS:

Seed cloves subjected to negative temperatures increased the quality of noble garlic cultivated in the semi-arid region.
Pre-treatment at -5 to -3 ºC for 50 days led to bulbs with lower soluble solids/titratable acidity ratio.
Negative temperatures produced bulbs with pungency and industrial index, good for fresh consumption and industrial processing.

ABSTRACT: Vernalization at subzero temperatures increases garlic productivity by artificially providing the necessary cold for the plants to fully develop in the field. However, it remains to be seen whether productivity increases occur with or without a loss in post-harvest bulb quality. Therefore, this study aimed to evaluate the effects of vernalization under negative temperatures for different periods on the quality characteristics of noble garlic grown in the Brazilian semi-arid region. Two experiments were conducted, one in Mossoró and another in São Miguel, RN, Brazil, evaluated separately, and outlined in randomized complete blocks. The treatments consisted of vernalization under three temperatures (-5 to -3 ºC, -2 to 0 °C, and 1 to 3 °C) and three periods (50, 55, and 60 days) of the garlic seed clove, with four replications. At harvest time, the bulbs were evaluated for their post-harvest quality. In both experiments, treatment with a temperature of -5 to -3 ºC for 50 days led to bulbs with a lower soluble solids/titratable acidity ratio, with values of 4.1 in Mossoró and 4.3 in São Miguel. In the Mossoró experiment, the temperature of -5 to -3 °C, associated with the period of 55 days, promoted the highest levels of soluble solids (38.94%). Negative temperatures and times of 50 and 55 days increased the pyruvic acid content of garlic grown in São Miguel, 85.36 and 83.98 µMoles of pyruvic acid, respectively. Vernalization at temperatures below zero resulted in bulbs with good characteristics for fresh consumption and industrial processing.

Key words: *Allium sativum*, titratable acidity, pungency, pyruvic acid

RESUMO: A vernalização em temperaturas abaixo de zero aumenta a produtividade do alho, fornecendo artificialmente o frio necessário para que as plantas se desenvolvam plenamente no campo. No entanto, ainda não se sabe se os aumentos de produtividade ocorrem com ou sem perda na qualidade pós-colheita dos bulbos. Portanto, este estudo teve como objetivo avaliar os efeitos da vernalização sob temperaturas negativas por diferentes períodos nas características de qualidade do alho nobre cultivado no semiárido brasileiro. Dois experimentos foram conduzidos, um em Mossoró e outro em São Miguel, RN, Brasil, avaliados separadamente e delineados em blocos casualizados completos. Os tratamentos consistiram na vernalização sob três temperaturas (-5 a -3 ºC, -2 a 0 °C e 1 a 3 °C) e três períodos (50, 55 e 60 dias) do alho semente, com quatro repetições. No momento da colheita, os bulbos foram avaliados quanto à sua qualidade pós-colheita. Em ambos os experimentos, o tratamento com temperatura de -5 a -3 ºC por 50 dias produziu bulbos com menor relação sólidos solúveis/acidez titulável, com valores de 4,1 em Mossoró e 4,3 em São Miguel. No experimento de Mossoró, a temperatura de -5 a -3 °C, associada ao período de 55 dias, promoveu os maiores teores de sólidos solúveis (38,94%). As temperaturas negativas, bem como os tempos de 50 e 55 dias, aumentaram os teores de ácido pirúvico do alho cultivado em São Miguel, 85,36 e 83,98 µMoles de ácido pirúvico, respectivamente. A vernalização em temperaturas abaixo de zero produziu bulbos com boas características para consumo in natura e processamento industrial.

Palavras-chave: *Allium sativum*, acidez titulável, pungência, ácido pirúvico

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* Corresponding author - E-mail: ericmoraais@gmail.com
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Introduction

Garlic has great economic and social importance for small producers in Brazilian family farming. It is known worldwide for its use in cooking, fresh consumption, and industrialized products (Santos et al., 2022). Furthermore, garlic is known to have medicinal and nutraceutical properties, such as vitamins, antioxidants, minerals, organosulfur compounds and others, which promote health benefits by reducing the risk of cancer and blood glucose levels, and help in controlling blood pressure, for example (Atif et al., 2020).

Despite its great relevance, Brazilian garlic production is insufficient to meet domestic market demand, requiring almost 45% of the garlic consumed to be imported from other countries (Morais et al., 2023). However, in recent years, the production of this vegetable in the country has been gradually increasing, mainly due to the use of technologies that enabled the expansion of noble garlic cultivation to other regions, producing bulbs with the necessary quality to compete with imported garlic.

Vernalization constitutes a key technology that expands both the planting area and the growing season of high-quality garlic cultivars, previously restricted to southern Brazil (Morais et al., 2023). This technique involves exposing seed garlic to low temperatures for prolonged periods. Reports in the literature primarily describe seed garlic vernalization at positive temperatures ranging from 0 to 5 °C, consistently increasing the productivity of noble cultivars, including under semi-arid conditions (Resende et al., 2011; Lopes et al., 2016a; Dong et al., 2019; Azmi et al., 2022).

Low temperatures applied during the pre-planting treatment of garlic seed alter the accumulation of proteins associated with cell growth, antioxidant activity, macromolecule transport, protein folding, and transcriptional regulation (Dufoo-Hurtado et al., 2015; Lucena et al., 2016a). Recent studies by Luz et al. (2022, 2023) and Morais et al. (2023) demonstrate that vernalization at subzero temperatures also enhances the productivity of noble cultivars, indicating that negative temperatures can elicit physiological responses beneficial to the development of noble garlic.

However, knowledge about the effects of vernalization at negative temperatures on post-harvest bulb quality remains limited. Characteristics such as pungency, total solids, and industrial index play a decisive role in consumer acceptance of garlic for both fresh and industrial uses. Therefore, in addition to achieving high productivity, producers must ensure bulbs with appropriate physical and chemical attributes after harvest.

In this context, this study aimed to evaluate the effects of vernalization at negative temperatures for different durations on the quality of noble garlic cultivated in the Brazilian semi-arid region.

Material and Methods

Experiments were carried out simultaneously between March and October 2022. In Mossoró, RN, the experiment was conducted at the Rafael Fernandes Experimental Farm, belonging to the Federal Rural University of the Semi-Arid Region (5° 03' 37" S latitude and 37° 23' 50" W longitude, 72 m altitude). According to Köppen's classification, the region's climate is BSh. The average temperature throughout the experiment was 25.8 °C, with a minimum of 22.4 °C and a maximum of 28.1 °C, with an average relative humidity of 80% (Figure 1A). A meteorological station near the study area (approximately 1.0 km) monitored rainfall, relative humidity, and air temperature data. The soil at the Experimental Farm is classified as Ultisol (United States, 2014), which corresponds to *Argissolo* in the Brazilian Soil Classification System (Santos et al., 2018).

In São Miguel, RN, the experiment was conducted at SGagro Farm (6º 16' 01" S latitude and 38° 38º 31' 45" W longitude, 602 m altitude). According to Köppen's classification, the region's climate is Aw. The average temperature was 23.8 °C, varying between 20.4 and 27.1 °C, with an average relative humidity of 70% (Figure 1B), during the months of the field experiment. Climatic data was monitored using a meteorological station close to the experimental area (approximately 1.0 km). The soil at the SGagro Farm is classified as Inceptisol (United States, 2014), which corresponds to *Cambissolo* in the Brazilian Soil Classification System (Santos et al., 2018).

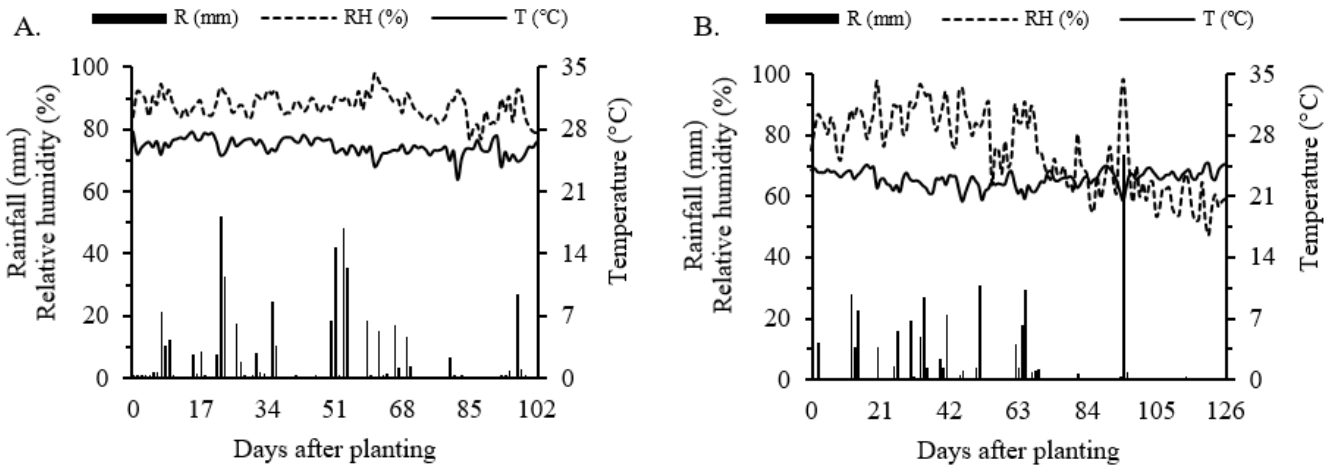

Figure 1. Rainfall (R), relative humidity (RH), and air temperature (T) average values in the municipalities of Mossoró (A) and São Miguel (B), RN, between May and September 2022

The soil chemical and physical characterization in the experimental areas, at 0-20 cm, was carried out following the methodology described by Teixeira et al. (2017) before implementing the experiments. The chemical and physical attributes are described in Table 1.

The experimental design was randomized complete blocks in a 3 × 3 factorial scheme with four replications. The treatments consisted of vernalization under three temperatures (-5 to -3, -2 to 0, and 1 to 3 °C) and three periods (50, 55, and 60 days) of the garlic seed clove. Each experimental plot had a total area of 1.80 m^2, consisting of 90 plants in six rows, with a 0.20 × 0.10 m spacing. The four central rows were considered the data collection area, disregarding the two plants at each end of the plot.

Studies on vernalization at subzero temperatures are still recent and limited, which restricts the availability of well-validated parameters. The temperature ranges used in this study (-5 to -3 °C, -2 to 0 °C, and 1 to 3 °C) were chosen based on preliminary results and the need to gradually assess the effects of subzero temperatures while minimizing damage to garlic seed tissues. More extreme temperatures, such as -10 °C, pose risks of freezing and cell death, whereas ranges closer to 0 to 2 °C approximate conventional vernalization conditions. Therefore, the treatments selected aimed to balance physiological safety with the investigation of extreme cold effects.

Garlic seed cloves' vernalization was carried out in a freezer, with the temperature controlled according to the treatments and relative humidity ranging from 60 to 70%. For planting to occur on the same date, the bulbs were stored in freezers at different times, according to pre-established periods. The garlic seed clove used in the experiments was the Ito cultivar (noble garlic), characterized by bulbs with white outer tunics and cloves with purplish skin, usually ranging from 8 to 12 cloves per bulb. This cultivar has good yield potential and high commercial quality.

For vernalization to occur, garlic cloves used as seed must be exposed to low temperatures for a certain period. Previous studies have sought to determine both the optimal vernalization temperature (ranging from 0 to 5 °C) and duration (e.g., 50, 55, or 60 days). In this study, in addition to temperature, the objective was to identify the appropriate duration, which may differ from previously reported conditions, as lower temperatures may accelerate or delay metabolic changes.

Table 1. Chemical and physical characterization of soils in experimental areas, at 0-20 cm depth

Layer (0-20 cm)	Unit	Mossoró	São Miguel
Chemical attributes			
pH	H_2O	6.18	7.33
EC	dS m^{-1}	0.15	0.56
O.M.	g kg^{-1}	8.5	18.9
P	mg dm^{-3}	8.4	23.6
K^+		0.12	0.96
Ca^{2+}		1.25	6.84
Mg^{2+}		0.64	2.18
Al^{3+}	$cmol_c$ dm^{-3}	0	0
H+Al		0.7	0.43
SB		2.01	10.02
T		2.71	10.45
V	%	74.12	95.88
Particle size analysis			
Sand		90	53.8
Clay	%	5	31
Silt		5	15.2

EC - Electrical conductivity; O.M. - Organic matter; SB - Sum of bases; T - Cation exchange capacity at pH 7.0; V - Base saturation

Soil preparation of the experimental areas was performed conventionally, with plowing and harrowing, followed by the raising of the beds. Fertilization in both experiments was based on soil analysis, following the recommendation of Holanda et al. (2017). In the experiment in Mossoró, 15 t ha^{-1} of organic compost and 180 kg ha^{-1} of P_2O_5 were used in planting, and in São Miguel, 15 t ha^{-1} of organic compost, 180 kg ha^{-1} of P_2O_5, 20 kg ha^{-1} of N, 40 kg ha^{-1} of K_2O, 112 kg ha^{-1} of Ca, 73 kg ha^{-1} of S, 12 kg ha^{-1} of Zn and 1.65 kg ha^{-1} of B were used in planting. The fertilizers used in the basal fertilization were formulated NPK (6-24-12), single superphosphate (18% P_2O_5, 16% Ca and 10% S), zinc sulfate (20% Zn and 10% S), and boric acid (17% B).

Top dressing was carried out weekly via fertigation, starting at 22 days and ending at 90 days after planting (DAP), totaling 10 applications during this period. Fertigation was performed by drip, with three hoses installed per block, emitters spaced every 0.30 m, and a flow rate of 1.5 L h^{-1}. In total, 79.0 kg ha^{-1} of N, 60.0 kg ha^{-1} of K_2O, 26.0 kg ha^{-1} of S, 14.3 kg ha^{-1} of Mg, 5.0 kg ha^{-1} of Ca, 13.5 kg ha^{-1} of Zn, 2.1 kg ha^{-1} of B and 0.60 kg ha^{-1} of the commercial formulation Rexolin® BRA (11.6% of K_2O, 1.28% of S, 0.86% of Mg, 2.1% of B, 0.36% of Cu, 2.66% of Fe, 2.48% of Mn, 0.036% of Mo and 3.38% of Zn) were applied. The sources of fertilizers used were urea (45% N), potassium nitrate (13% N and 44% K_2O), calcium nitrate (14% N and 16% Ca), magnesium sulfate (9% Mg and 12% S), zinc sulfate (20% Zn and 10% S), potassium chloride (60% K_2O), and boric acid (17% B).

After the vernalization period, the bulbs were threshed and planted at 2 to 3 cm depth. A sprinkler irrigation system was used, with micro-sprinklers spaced every 3.0 m and a flow rate of 27 L h^{-1}. The irrigation depths were calculated according to the soil type, crop development stage, and the region's climate (Allen et al., 2006).

During the experiments, manual weeding was performed to control spontaneous vegetation. Spays were carried out to prevent and control purple blotch disease and bacterial diseases using products based on dithiocarbamate, difenoconazole, azoxystrobin, and copper oxychloride. Regarding pest control, such as thrips and caterpillars, insecticides based on chlorfenapyr, imidacloprid, and beta-cyfluthrin were used.

Garlic was harvested when approximately 2/3 of the plants' leaves were yellow or dry. In the Mossoró experiment, the bulbs were harvested at 78 and 102 DAP for treatments with positive and negative vernalization temperatures, respectively. Following the same pattern, the garlic harvest in São Miguel was carried out at 89 DAP for vernalization with positive temperatures and at 126 DAP for treatments with negative temperatures.

After harvesting, the bulbs' curing process began. The plants remained in the field in full sun for three days. Subsequently, they were placed in a room and cured in the shade for 12 days. Bulb cleaning was carried out by eliminating dry and

dirty roots, leaves, and tunics. After cleaning, the bulbs were classified based on transverse diameter into commercial bulbs (transverse diameter greater than or equal to 32 mm) and non-commercial bulbs (transverse diameter less than 32 mm). The equipment used to classify the garlic bulbs was made of wood, with progressively sized openings arranged in sequence. This system allowed the separation of the bulbs into the previously established diameter classes.

For post-harvest characterization of garlic, 20 bulbs classified as commercial were used. Of these, ten were processed to obtain a homogeneous paste, and the hydrogen potential (pH), titratable acidity (mEq H_3O^+ $100g^{-1}$) (IAL, 2005), soluble solids (%) (AOAC, 2002), and soluble solids/titratable acidity ratio were determined. Total soluble sugars (%) were quantified by the anthrone method (Yemm & Willis, 1954), whereas the pungency analysis (µMoles of pyruvic acid mL^{-1} of juice) was performed by the colorimeter method described by Schwimmer & Weston (1961).

To determine the total solids content, a sample of 10 commercial bulbs was weighed (fresh mass), and then these bulbs were dried in a forced air circulation oven at 65 ºC until they reached constant weight (dry mass); total solids (%) was calculated by dividing the dry mass by the fresh mass of the sample, multiplying the result by 100 (IAL, 2008). The industrial index (II) was determined by multiplying the total solids by pyruvic acid, divided by 100.

The normality of the data was confirmed to ensure the validity of the results. The data were then subjected to analysis of variance (ANOVA) separately for each experiment, with the means compared by Tukey's test ($p \leq 0.05$), using the statistical program SISVAR®, version 5.4 (Ferreira, 2019).

Results and Discussion

In the Mossoró experiment, vernalization of garlic seed clove at -5 to -3 °C promoted the highest levels of soluble solids (38.94%) when associated with a period of 55 days, statistically equal to the 60-day period (38.26%). When evaluating the effect of temperature within each vernalization period, we found that at 50 days, there was no difference between temperatures, whereas at 55 and 60 days of vernalization, higher soluble solids content was obtained at -5 to -3 °C. In São Miguel, the highest levels were observed at positive temperatures, not differing between vernalization periods. However, among the periods analyzed, vernalization for 50 days, at the temperature of 1 to 3 ºC, led to the highest average soluble solids content (39.41%) (Table 2).

The Mossoró experimental area is located in a lower altitude region (72 m), with high temperatures throughout the development of the garlic crop (Figure 1A). These conditions negatively influenced bulb production (Li et al., 2020; Morais et al., 2023; Pereira et al. 2025), due to the high temperatures in the region (Golla, 2021; Oliveira et al., 2024). However, treatments with negative temperatures produced bulbs with higher soluble solids levels than those under positive temperatures (Table 2). This is a promising result for garlic grown in Mossoró, showing that vernalization temperatures act positively under more stressful conditions for plants, resulting in bulbs with higher levels of soluble solids.

In São Miguel, on the other hand, the positive temperature treatment generated bulbs with higher levels of soluble solids (Table 2). This was probably because the experimental area is located in a higher altitude (600 m) region, providing mild temperatures throughout the crop cycle (Figure 1B). These conditions favor the development of noble garlic plants (Luz et al., 2022). Thus, vernalization under positive temperature was sufficient to produce bulbs with higher soluble solids levels.

In garlic produced in Mossoró, vernalization at temperatures of -5 to -3 °C (6.74) and -2 to 0 °C (6.73) promoted higher pH values at 50 days, as well as at -5 to -3 °C at 55 days (6.73). For garlic grown in São Miguel, the highest pH occurred in the treatment with positive temperature for 60 days (6.76) (Table 2).

Table 2. Soluble solids and hydrogen potential of noble garlic, cv. Ito, obtained from seed cloves subjected to different temperatures and periods

Period (days)	Soluble solids (%)			Hydrogen potential		
	Temperature (ºC)			Temperature (ºC)		
	-5 to -3	-2 to 0	1 to 3	-5 to -3	-2 to 0	1 to 3
			Mossoró			
50	37.63 ± 0.07^{SE} aB	36.90 ± 0.14 aA	37.28 ± 0.14 aA	6.74 ± 0.02 aA	6.73 ± 0.01 aA	6.69 ± 0.01 bA
55	38.94 ± 0.24 aA	36.93 ± 0.25 bA	37.00 ± 0.22 bA	6.73 ± 0.00 aA	6.70 ± 0.01 abA	6.69 ± 0.00 bA
60	38.26 ± 0.31 aAB	37.20 ± 0.09 bA	36.81 ± 0.36 bA	6.69 ± 0.01 aB	6.70 ± 0.01 aA	6.71 ± 0.01 aA
F_T		28.98**			5.05*	
F_P		1.73^{ns}			3.12^{ns}	
$F_{T\times P}$		3.84*			4.47**	
CV (%)		1.25			0.28	
			São Miguel			
50	37.40 ± 0.19 bA	35.35 ± 0.43 cB	39.41 ± 0.59 aA	6.70 ± 0.01 bA	6.70 ± 0.01 bA	6.73 ± 0.01 aB
55	36.53 ± 0.07 bA	37.39 ± 0.37 bA	38.86 ± 0.35 aA	6.68 ± 0.01 bA	6.68 ± 0.01 bA	6.75 ± 0.01 aAB
60	36.51 ± 0.06 bA	37.36 ± 0.40 bA	38.85 ± 0.41 aA	6.67 ± 0.01 bA	6.68 ± 0.01 bA	6.76 ± 0.01 aA
F_T		41.47**			49.68**	
F_P		0.31^{ns}			0.56^{ns}	
$F_{T\times P}$		6.69**			3.43*	
CV (%)		1.89			0.27	

*,**, ns - Significant at $p \leq 0.05$ and at $p \leq 0.01$ and not significant by F test. Means followed by the same uppercase letter in the column and lowercase letter in the line do not differ according to Tukey's test. SE - Standard error. F_T - F calculated for temperature; F_P - F calculated for period; $F_{T\times P}$ - F calculated for interaction between temperature and period; CV - Coefficient of variation

Cultivation in regions with higher temperatures, as observed in Mossoró, whose average temperature throughout the experiment was 25.8 °C (Figure 1A), reduces the temperature and vernalization period necessary to produce bulbs with higher pH values. In São Miguel, conversely, milder temperatures throughout plant development, with an average of 23.5 ºC (Figure 1B), increase the temperature and vernalization period necessary to obtain bulbs with higher pH (Table 2).

Noble garlic requires low temperatures and a long photoperiod (Luz et al., 2022) throughout the cultivation cycle to fully develop (Pereira et al., 2021). According to Morais et al. (2023), the climate influences garlic production, with lower productivity in places with high temperatures. This indicates that climatic factors condition the development of the crop and, consequently, the quality of the bulbs. According to Atif et al. (2020), a small increase in temperature (1 to 2 ºC) influences the final yield of the garlic crop.

Negative vernalization temperatures favored the production of bulbs with higher titratable acidity. The vernalization period also affected this characteristic, so a shorter period resulted in garlic with higher titratable acidity, regardless of the temperature used. The highest titratable acidity values, 9.18 and 8.17 mEq H_3O^+ $100g^{-1}$, in Mossoró and São Miguel, respectively, were observed when vernalizing garlic seed cloves at -5 to -3 ºC for 50 days (Table 3).

At all temperatures analyzed in Mossoró and São Miguel, a shorter vernalization time was required to produce bulbs with greater titratable acidity. For this characteristic, the treatment with a temperature of -5 to -3 ºC in both locations stood out, producing more acidic bulbs (Table 3). These findings suggest a higher concentration of organic acids dissolved in the vacuoles of the cells, which is an indication to evaluate the aroma of garlic since higher acidity results in more aromatic and flavorful bulbs (Lopes et al., 2016b; Lucena et al., 2016b). Thus, bulbs with this characteristic are desirable for industrial processing and fresh consumption.

As the vernalization temperature and period increased, the bulbs' soluble solids/titratable acidity ratio increased (Table 3). The 1 to 3 ºC temperature for 60 days increased the ratio between soluble solids and titratable acidity in Mossoró (5.53) and São Miguel (6.69).

In both study sites, to obtain bulbs with higher soluble solids/titratable acidity ratios, the seed garlic had to be treated at a higher temperature (1 to 3 ºC) and for longer (60 days). On the other hand, the combination of the lowest temperature (-5 to -3 ºC) and the shortest vernalization period (50 days) led to a lower ratio between these variables (Table 3). This behavior was inverse to that observed with titratable acidity (Table 2).

The soluble solids/titratable acidity ratio is one of the best ways to assess the post-harvest quality of vegetables (Lima et al., 2019). It describes the balance between sugars and organic acids content, which can be used as a criterion for evaluating aroma and flavor. In the case of garlic, lower ratios between soluble solids/titratable acidity indicate higher levels of some organic acids, giving a more intense aroma to this vegetable, which is a characteristic desired by consumers. Both experiments showed this behavior when vernalizing the garlic seed clove at -5 to -3 ºC for 50 days.

The interaction between the vernalization temperature and period factors influenced the total soluble sugar content of garlic grown in Mossoró. The lowest temperature (-5 to -3 °C) provided the highest total soluble sugar content, regardless of the period. In this range, the 55-day period promoted a higher content of total soluble sugars in the bulbs (38.51%) (Table 4).

The total soluble sugar levels observed in the São Miguel experiment was significantly affected by the factors, vernalization temperature and period individually. The highest total soluble sugar level (29.63%) was obtained with vernalization carried out over 60 days, significantly similar to the 55 days of vernalization (28.35%). Regarding temperatures, the highest content of total soluble sugars was observed in the treatment of -5 to -3 ºC (31.25%) (Table 4).

Table 3. Titratable acidity and soluble solids/titratable acidity ratio of noble garlic, cv. Ito, obtained from seed cloves subjected to different temperatures and periods

Period (days)	Titratable Acidity (mEq H_3O^+/100g of paste)			Soluble solids/titratable acidity		
	Temperature (ºC)			Temperature (ºC)		
	-5 to -3	-2 to 0	1 a 3	-5 to -3	-2 to 0	1 to 3
			Mossoró			
50	9.19 ± 0.09[SE] aA	8.49 ± 0.08 bA	7.50 ± 0.08 cA	4.10 ± 0.04 cC	4.35 ± 0.04 bB	4.97 ± 0.04 aC
55	8.38 ± 0.06 aB	8.25 ± 0.08 aB	7.17 ± 0.06 bB	4.65 ± 0.01 bB	4.48 ± 0.03 bB	5.16 ± 0.04 aB
60	7.85 ± 0.07 aC	7.59 ± 0.07 bC	6.67 ± 0.10 cC	4.87 ± 0.05 bA	4.90 ± 0.04 bA	5.53 ± 0.13 aA
F_T		364.21**			158.95**	
F_P		192.17**			108.22**	
$F_{T\times P}$		6.73**			4.72**	
CV (%)		1.62			2.2	
			São Miguel			
50	8.71 ± 0.18 aA	7.26 ± 0.10 bA	6.34 ± 0.03 cA	4.30 ± 0.09 cB	4.88 ± 0.12 bB	6.22 ± 0.13 aB
55	8.23 ± 0.14 aB	6.49 ± 0.02 bB	6.12 ± 0.03 cA	4.45 ± 0.07 cB	5.76 ± 0.07 bA	6.35 ± 0.05 aB
60	7.37 ± 0.13 aC	6.27 ± 0.08 bB	5.66 ± 0.12 cB	4.96 ± 0.10 cA	5.96 ± 0.10 bA	6.89 ± 0.22 aA
F_T		334.65**			215.83**	
F_P		75.32**			37.75**	
$F_{T\times P}$		4.79**			3.66**	
CV (%)		2.89			4.09	

*,**, ns - Significant at $p \leq 0.05$ and at $p \leq 0.01$ and not significant by F test. Means followed by the same uppercase letter in the column and lowercase letter in the line do not differ according to the Tukey's test. SE - Standard error. F_T - F calculated for temperature; F_P - F calculated for period; $F_{T\times P}$ - F calculated for interaction between temperature and period; CV - Coefficient of variation

Table 4. Total soluble sugars and pungency of noble garlic, cv. Ito, obtained from seed cloves subjected to different temperatures and periods

Mossoró[1]						
Period (days)	Total soluble sugars (%)			Pungency (μMoles of pyruvic acid mL of juice^{-1})		
	Temperature (ºC)			Temperature (ºC)		
	-5 to -3	-2 to 0	1 a 3	-5 to -3	-2 to 0	1 to 3
50	31.98 ± 0.43^{SE} aC	27.65 ± 0.84 bA	22.46 ± 0.27 cAB	90.42 ± 0.58 aA	85.77 ± 1.11 bB	87.10 ± 0.72 bB
55	38.51 ± 0.14 aA	26.64 ± 0.25 bA	23.13 ± 0.72 cA	81.28 ± 0.29 bB	90.35 ± 1.04 aA	92.72 ± 1.96 aA
60	34.05 ± 0.44 aB	26.59 ± 0.14 bA	21.06 ± 0.36 cB	81.08 ± 0.53 cB	86.75 ± 0.75 bB	91.46 ± 1.21 aA
F_T	574.68**			36.53**		
F_P	21.39**			3.04^{ns}		
$F_{T\times P}$	18.98**			25.95**		
CV (%)	3.29			2.02		
São Miguel[2]						
Period (days)	Total soluble sugars (%)			Pungency (μMoles of pyruvic acid mL^{-1})		
50	26.01 ± 0.97 b			85.36 ± 2.04 a		
55	28.35 ± 0.89 a			83.98 ± 2.16 a		
60	29.63 ± 0.84 a			81.43 ± 2.07 b		
Temperature (ºC)	Total soluble sugars (%)			Pungency (μMoles of pyruvic acid mL^{-1})		
-5 to -3	31.25 ± 0.61 a			88.62 ± 0.85 a		
-2 to 0	27.99 ± 0.63 b			87.85 ± 0.87 a		
1 to 3	24.75 ± 0.60 c			74.30 ± 0.72 b		
F_T	54.60**			193.45**		
F_P	17.36**			11.81**		
$F_{T\times P}$	0.83^{ns}			1.76^{ns}		
CV (%)	5.45			2.4		

*,**, ns - Significant at $p \leq 0.05$ and at $p \leq 0.01$ and not significant by F test. [1]Means followed by the same uppercase letter in the column and lowercase letter in the line do not differ according to the Tukey's test; [2] Means followed by the same lowercase letter in the column do not differ according to the Tukey's test. SE - Standard error. F_T - F calculated for temperature; F_P - F calculated for period; $F_{T\times P}$ - F calculated for interaction temperature and period; CV - Coefficient of variation

Garlic is a vegetable rich in sugars, with percentages above 20% (Lucena et al., 2016b). The increase in total soluble sugars in the treatments under vernalization at negative temperatures in both experiments may be associated with the increase in the crop cycle. By spending more time in the field, the plant accumulates more photoassimilates (Morais et al., 2023), including starch, which can be converted into soluble sugars.

High sugar contents, especially reducing sugars, such as glucose and fructose, are valued for the production of black garlic (Lima et al., 2019). This type of garlic is obtained from a fermentation process, in which the bulb is subjected to controlled temperature and humidity conditions, and, in the end, its color darkens (Vitor et al., 2023). Bulbs with high sugar contents are more desirable for producing this type of garlic since, at the end of industrial processing, the garlic produced will have a more pronounced sweet flavor. Thus, in both experimental locations, the highest sugar levels were observed when treating seed garlic at negative temperatures, again highlighting the potential of these treatments to increase the quality of noble garlic.

In Mossoró, the interaction between vernalization temperature and period influenced the bulbs' pungency. The positive temperature for 55 days resulted in bulbs with greater pungency (92.72 μMoles of pyruvic acid mL of juice^{-1}), not significantly different from 60 days. At temperatures of -5 to -3 ºC and -2 to 0 ºC, for 50 and 55 days, respectively, the plants also produced bulbs with high levels of pyruvic acid (90.42 and 90.35 μMoles mL of juice^{-1}) (Table 4).

For the pungency of the bulbs, in the São Miguel experiment, there was a significant effect of the vernalization period and temperature factors separately. The period of 50 days led to bulbs with greater pungency (85.36 μMoles of pyruvic acid mL of juice^{-1}), statistically equal to 55 days (83.98 μMoles of pyruvic acid mL of juice^{-1}). Regarding temperatures, treatment at -5 to -3 ºC resulted in bulbs with a higher content of pyruvic acid (88.62 μMoles of pyruvic acid mL of juice^{-1}), which did not differ statistically from treatment at -2 to 0 °C (87.85 μMoles of pyruvic acid mL of juice^{-1}) (Table 4).

In both Mossoró and São Miguel, the average temperatures throughout the cultivation also influenced how vernalization acted on the pungency of the bulbs. The higher temperatures throughout the Mossoró experiment increased the temperature and the vernalization period necessary to produce the bulbs with greater pungency. On the other hand, the milder temperatures of São Miguel reduced the temperature and vernalization period required by the crop to produce bulbs with higher concentrations of pyruvic acid (Table 4).

The high levels of pyruvic acid observed in the bulbs produced in Mossoró may be due to the interaction between the genotype and the environment. According to Vargas et al. (2010), the interaction between these factors strongly influences the pyruvic acid content in the bulbs. Thus, garlic grown under warmer climatic conditions produces bulbs with greater pungency. Other studies carried out in the semi-arid region of the country have also reported high levels of pyruvic acid in garlic bulbs (Lopes et al., 2016b; Lucena et al., 2016b; Lima et al., 2019; Bessa et al., 2020).

The typical pungency of garlic is related to the concentration of pyruvic acid present in the bulbs. Thus, quantifying pyruvic acid content is reliable for estimating garlic pungency (Thangasamy et al., 2021). For Prakash & Prasad (2023), garlic can be classified based on pyruvic acid content into three groups, namely low pungency garlic (<40 μmol mL^{-1}), medium pungency garlic (40 - 70 μmol mL^{-1}) and high pungency garlic (> 70 μmol mL^{-1}).

All values of pyruvic acid observed in this study are above 70 µmol mL^{-1}, so the garlic produced is classified as of high pungency. High levels of pyruvic acid in the bulbs provide better flavor and aroma for this vegetable (Lima et al., 2019). Therefore, bulbs with this characteristic have greater acceptance in the consumer market, whether in natural or industrialized form.

The vernalization temperatures influenced the total solids content of the noble garlic. In the Mossoró and São Miguel experiments, treatments with positive temperature produced bulbs with higher total solids contents (37.5 and 39.77%), respectively (Table 5).

Garlic seed cloves treated with a positive vernalization temperature resulted in bulbs with higher total solids (Table 5). It is important to use bulbs with high total solids for the industrialization of garlic, especially when dehydrated. This occurs because the amount of product obtained from the dehydration process largely depends on the total solids content present in the raw material (Bessa et al., 2017). Thus, the higher the total solids content of the bulbs, the higher the industrial yield of garlic (Lopes et al., 2016b; Lucena et al., 2016b).

It is desired that the total solids in the garlic crop are above 30% to have a satisfactory industrial yield (Bessa et al., 2017). Furthermore, bulbs with these conditions have better post-harvest conservation capacity (Lopes et al., 2016b). Therefore, regardless of the seed garlic treatment, all bulbs produced in the present work have the potential for industrial processing and good prospects for post-harvest storage.

The industrial index of garlic grown in Mossoró was influenced by the interaction between temperature and period. The positive vernalization temperature, associated with the 55 days, provided bulbs with a higher industrial index (34.76). High values were also observed with temperatures of -5 to -3 ºC for 50 days and -2 to 0 ºC for 55 days, 32.50 and 33.41, respectively (Table 6).

In the São Miguel experiment, the industrial index was significantly affected by the vernalization temperature factor individually. Treatments with temperatures of -5 to -3 °C and -2 to 0 °C produced bulbs with higher industrial indices, 31.59 and 31.66, respectively (Table 6).

The industrial index is important in producing dehydrated garlic (Lima et al., 2019). This characteristic is influenced by the total solids and pyruvic acid content of the bulbs (Lucena et al., 2016b; Bessa et al., 2020), so for a better acceptance of this vegetable as a condiment, bulbs need to have high values of the industrial index. As a result, the bulbs produced

Table 5. Total solids (%) of noble garlic, cv. Ito, obtained from seed cloves subjected to different temperatures and periods

Temperature (ºC)	Mossoró	São Miguel
	Total solids (%)	
-5 to -3	36.31 ± 0.29^{SE} b	35.66 ± 0.53 b
-2 to 0	36.98 ± 0.22 ab	36.04 ± 0.69 b
1 to 3	37.50 ± 0.17 a	39.77 ± 0.34 a
F_T	5.89**	17.72**
F_P	0.26^{ns}	0.99^{ns}
F_{TxP}	0.49^{ns}	0.66^{ns}
CV (%)	2.32	5.03

*,**, ns - Significant at p ≤ 0.05 and at p ≤ 0.01 and not significant by F test. Means followed by the same lowercase letter in the column do not differ according to the Tukey's test. SE - Standard error. F_T - F calculated for temperature; F_P - F calculated for period; $F_{T\times P}$ - F calculated for temperature and period interaction; CV - Coefficient of variation

Table 6. Industrial index of noble garlic, cv. Ito, obtained from seed cloves stored to different temperatures and periods

Mossoró			
Period (days)	Industrial Index		
	Temperature (ºC)		
	-5 to -3	-2 to 0	1 to 3
50	32.50 ± 0.58^{SE} aA	31.92 ± 0.44 aA	32.74 ± 0.56 aB
55	29.87 ± 0.51 bB	33.41 ± 0.71 aA	34.76 ± 0.96 aA
60	29.38 ± 0.10 cB	31.88 ± 0.33 bA	34.26 ± 0.48 aAB
F_{TV}	28.91**		
F_{PV}	1.88^{ns}		
$F_{TV\,x\,PV}$	7.17**		
CV (%)	3.33		
São Miguel			
Temperature (ºC)	Industrial Index		
-5 to -3	31.59 ± 0.49 a		
-2 to 0	31.66 ± 0.70 a		
1 to 3	29.55 ± 0.32 b		
F_T	5.59*		
F_P	0.40^{ns}		
F_{TxP}	0.48^{ns}		
CV (%)	5.69		

*,**, ns - Significant at p ≤ 0.05 and at p ≤ 0.01 and not significant by F test. Equal letters do not differ statistically according to the Tukey Test. SE - Standard error. F_T - F calculated for temperature; F_P - F calculated for period; $F_{T\times P}$ - F calculated for temperature and period interaction; CV - Coefficient of variation

in Mossoró, from the treatment with positive temperature, associated with the period of 55 days, had a better industrial index for producing dehydrated garlic. However, producing bulbs with high industrial index values was also possible using combinations of -5 to -3 ºC for 50 days and -2 to 0 ºC for 55 days (Table 6). These results show the potential of below-zero vernalization temperatures to produce bulbs with good characteristics for industrial processing.

The high industrial index observed in this study is associated with the use of dehydrated garlic by the industry, a characteristic considered desirable. It should be noted that the harvest was carried out at different times, since the optimal harvest point varied according to the treatments and cultivation sites. However, no excessive dehydration of the bulbs was observed during storage, as in all cases they were kept for the same period after harvest.

In the experiment of São Miguel, conversely, pre-treatment with negative temperatures resulted in bulbs with a higher industrial index, that is, bulbs with better characteristics for producing dehydrated garlic. This was due to the higher pyruvic acid levels (pungency) observed in these treatments (Table 4). According to Bessa et al. (2020), bulbs with high pungency have a higher industrial index, resulting in products with higher industrial yield and more pronounced aroma.

Generally, vernalization at negative temperatures increases the quality of noble garlic grown in the Brazilian semi-arid region. In Mossoró and São Miguel, bulbs with higher titratable acidity and lower total soluble solids/titratable acidity ratio were produced. These characteristics give the bulbs a more pronounced flavor and aroma, making this vegetable more attractive to the consumer. Vernalization at temperatures below zero in both cultivation sites also provided bulbs with higher levels of total soluble sugars, ideal for producing better-quality black garlic.

Mossoró and São Miguel's climate influenced how the garlic plants responded to vernalization. The experimental area of Mossoró is located in a region of lower altitude (72 m), with temperatures that are unfavorable to the development of garlic crops. However, vernalization with temperatures below zero acted positively under these conditions, resulting in bulbs with higher soluble solids. On the other hand, the São Miguel experiment is located in a region with a higher altitude (600 m), with temperatures more favorable to the cultivation of noble garlic. Under these conditions, vernalization under positive temperature was sufficient to produce bulbs with higher levels of soluble solids.

The climatic conditions of each cultivation environment also influenced how vernalization acted on the pungency of the bulbs. In Mossoró, the high temperatures throughout the experiment increased the temperature and the vernalization period necessary to produce the bulbs with greater pungency. On the other hand, in the São Miguel experiment, the mild temperatures throughout the cultivation cycle reduced the temperature and the vernalization period required by the crop to obtain bulbs with higher concentrations of pyruvic acid. In this case, reducing the vernalization period can be favorable from an economic point of view since it reduces production costs.

The vernalization of garlic seed cloves at low temperatures increases the quality of noble garlic produced in semi-arid regions. The effects of this technique on some post-harvest attributes of the bulbs vary according to the interaction between environmental characteristics and the temperature and period of vernalization. This indicates that, depending on the climatic conditions under which garlic is grown, the combination of temperature and a certain period of vernalization has different effects on the responses of garlic plants, affecting the quality of the bulbs.

Conclusions

1. The treatment of seed garlic with negative temperatures (-5 to -3 ºC) increased the quality of noble garlic cultivated in the Brazilian semi-arid region, resulting in bulbs with higher levels of titratable acidity and total soluble sugars.

2. In both experiments, the pre-treatment at -5 to -3 ºC for 50 days led to bulbs with lower soluble solids/titratable acidity ratio. For garlic grown in Mossoró, the temperature of -5 to -3 °C, associated with a period of 55 days, generated bulbs with high levels of soluble solids.

3. The negative temperatures and the periods of 50 and 55 days increased the pyruvic acid content of garlic grown in São Miguel.

4. Although the responses varied between Mossoró and São Miguel due to differences in altitude and climate, negative temperatures generally promoted higher sugar accumulation and pungency, enhancing the industrial index and contributing to greater commercial value of the bulbs.

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