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Effect of Menthol Treatment on the Sprouting and Quality of Potato Tuber

Ye Xu, Yang Qin, Qianqian Hou, Defu Niu and Qingmin Chen *

College of Food Science and Engineering, Shandong Agriculture and Engineering University, Jinan 250100, China; z2020044@sdaeu.edu.cn (Y.X.); z2019017@sdaeu.edu.cn (Y.Q.); houqianqian0420@163.com (Q.H.); niudefu0526@163.com (D.N.)

* Correspondence: qmchen1207@163.com

Abstract: This study used Dutch potatoes at the end of dormancy as a material to explore the impact of menthol (0.2 and 0.5 g/kg based on potato mass) treatment on sprouting inhibition and potato quality. The findings revealed that a menthol concentration of 0.5 g/kg effectively inhibited potato tuber sprouting and significantly reduced glucoside alkaloid production. After a storage period of 15 days, the sprouting percentage and glucoside alkaloid content of potatoes treated with 0.5 g/kg menthol were observed to be significantly lower at 4.17% and 68.63 mg/kg, respectively, compared to the control group which exhibited values of 100% and 282.01 mg/kg, respectively ($p < 0.05$). Throughout the storage period, 0.5 g/kg of menthol promoted respiration, reduced malondialdehyde production in potatoes, inhibited polyphenol oxidase activity, and slowed down tissue browning. Additionally, it mitigated the decline in starch and soluble protein content, inhibiting the accumulation of reducing sugars.

Keywords: potato tuber; menthol; sprouting inhibition; quality; glucoside alkaloids

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1. Introduction

Potato (*Solanum tuberosum* L.) is an annual plant belonging to the Solanaceae family. With a global production exceeding 300 million tonnes each year [1,2], potatoes hold considerable economic value due to their high yield and nutritional quality, playing a crucial role in ensuring food security, driving industrial poverty alleviation, and fostering regional economic revitalisation across all nations, especially in developing countries [3]. However, potatoes are highly susceptible to sprouting during storage and transport [4], accompanied by the production of toxins such as glucoside alkaloids [5]. According to statistics, the annual loss of potatoes due to sprouting accounts for about 20–25% of the total potato production [6], resulting in substantial economic losses and posing food safety hazards.

Currently, various methods are employed for inhibiting potato sprouting during storage, including chemical treatment, irradiation, phytohormone treatment, and plant essential oil treatment. Common chemicals used for this purpose include chlorpropham (CIPC) and maleic hydrazide. CIPC, the most widely utilised commercial potato sprout inhibitor, hinders tuber sprouting by affecting spindle formation and interfering with cell division in meristematic tissues [7]. However, the safety concerns related to pharmaceutical residues in CIPC pose a significant threat to human health [8], leading to its complete ban in the EU since 2020. Maleic hydrazide, also known as 3,6-dihydroxypyridazine, acts as a growth regulator with various functions, including inhibiting potato sprouting. Despite its common use in potato cultivation, many countries lack established maximum residue limits for maleic hydrazide in potatoes, limiting its application. Radiation treatments, though effective, are irreversible, expensive, and have low market acceptance.

In phytohormone treatment, ethylene is a primary agent used to inhibit potato sprouting by influencing carbohydrate metabolism [9]. However, the continuous introduction of

ethylene into the potato storage environment requires specialised equipment for sealing and introduction, leading to higher costs. Additionally, ethylene can enhance the activity of potato polyphenol oxidase (PPO), accelerating potato browning and adversely affecting both potato quality and subsequent processing quality [10].

Recently, the inhibitory effect of plant essential oils on potato sprouting has gained attention due to their status as pure natural extracts. Essential oils such as carvone, lemongrass oil, jasmine oil, and menthol have been studied. Oosterhaven et al. [11] found that carvone effectively inhibits sprouting in a potato bud growth model, and its inhibitory effect is reversible. Yuan et al. [12] demonstrated that lemongrass essential oil effectively inhibits potato sprouting while significantly impacting potato sugar metabolism, delaying the decline of starch and soluble protein content during storage and preserving the nutritional components of potato tubers. Huang et al. [13] investigated the effects of jasmine essential oil and menthol on sprouting inhibition and quality in fresh potatoes. However, the effectiveness of menthol in inhibiting potato sprouting at the end of dormancy and its impact on quality remain unexplored and require further study. Therefore, this study wanted to explore the effects of menthol on potato germination and quality.

To investigate the impact of different concentrations of menthol treatment on Dutch potatoes at the end of dormancy, the effects of two concentrations of menthol on potato sprouting rate, glucoside alkaloid content, and quality parameters during storage at room temperature were investigated. Physiological changes, browning, starch and reducing sugar content, as well as amino acid composition were analysed in order to provide a potential solution for sprout control.

2. Materials and Methods

2.1. Materials

The potatoes utilised in this study were the Dutch early maturing variety V7 sourced from the Guanzhuang Vegetable Wholesale Market in Zhoucun District, Zibo City. Harvested in July 2022 after a dormant period, these potatoes were subsequently transported to the Fruit and Vegetable Storage and Preservation Laboratory of Shandong Agriculture and Engineering University. Fresh potatoes, characterised by their uniform size, and devoid of mechanical damage or diseases, were meticulously chosen for the experimentation. Menthol ($C_{10}H_{20}O$, CAS 89-78-1, purity: 99%) was obtained from the Wuhan Jixin Yibang Biotechnology Co., Ltd. (Wuhan, China).

2.2. Treatments

Potato tubers were segregated into plastic baskets, with each group comprising six baskets, each containing 10 potatoes (approximately 5 kg per basket). Three distinct sets were arranged: Control CK (no menthol application), Group 1 treated with an effective concentration of 0.2 g/kg menthol, and Group 2 treated with 0.5 g/kg menthol. The menthol and an inert material mixture were uniformly applied to the potato surfaces.

All groups were stored at room temperature (25 °C) for 15 days, with samples collected at 3-day intervals. For analysis, three baskets from each group were allocated to assess sprouting percentage, sprouting index, weight loss, and respiration intensity. The remaining three baskets were utilised for determining additional indices, including moisture content, browning degree, malondialdehyde (MDA) content, PPO activity, starch content, and soluble protein content.

To ensure sampling uniformity, the four-quadrant method was employed. Ten potato tubers from each treatment group were selected for peeling and sampling, then pulverised into powder using a liquid nitrogen grinding instrument, and the samples were subsequently preserved in an ultra-low temperature refrigerator at −80 °C for future analysis. The determination of all indicators was repeated three times for each sample.

2.3. Determination of Sprouting Percentage and Sprouting Index

The sprouting percentage and sprouting index were assessed at 3-day intervals (refer to the method of Yuan et al. [12] for the determination of sprouting percentage). Ten potatoes were randomly selected from each treatment, and the length of the longest sprout was measured using a vernier calliper. Sprouts with a length less than 2 mm were classified as non-sprouting or in an initial sprouting state (refer to the method of Şanli et al. [14] for the determination of sprouting index). Ten potatoes were randomly chosen for each treatment. The number of buds on each tuber was documented, and the length of the longest bud was measured with a vernier calliper and then averaged. The bud lengths were categorised into eight grades (Table 1), and the sprouting percentage (%) was computed as the numbers of sprouted tubers/total number of tubers × 100%; and the sprouting index (%) was computed as the sum of the length of sprouted tubers/(total number of tubers × highest length) × 100%.

Table 1. Sprouting grading criteria.

Bud Length/mm	Sprouting Grade	Bud Length/mm	Sprouting Grade
0~2	0	15~20	4
2~5	1	20~25	5
5~10	2	25~30	6
10~15	3	>30	7

2.4. Determination of Glucoside Alkaloids Content

The extraction method of glucoside alkaloids followed slight modifications of Oishi et al.'s [15] procedure: we recovered 5.0 g samples after grinding with liquid nitrogen, then mixed them with 30 mL of acidified acetonitrile ((acetonitrile:formic acid = 99:1):water = 5:1, v:v), vortexed and oscillated for 5 min. Then, sodium acetate (1.0 g) and anhydrous magnesium sulphate (2.0 g) were added, followed by another vortexing and oscillation for 5 min before centrifugation at a rate of 10,000× *g* for 10 min. The supernatant was filtered through a 0.45 μm organic membrane filter. The detection of α-solanine and α-chaconine was performed using HPLC (Shimadzu Corporation, Tokyo, Japan).

Separation occurred on a C_{18} column (Waters Corporation; dimensions: 4.6 mm × 250 mm; particle size: 5 μm), employing a mixture consisting of dipotassium hydrogen phosphate solution (pH adjusted to 7.6 with potassium dihydrogen phosphate; concentration: 0.1 mol L^{-1}), acetonitrile, and water in a ratio of l:6:3 as the mobile phase. A stepwise gradient program was used for isocratic elution. The injection volume for all samples was 10 μL. The flowrate was set at l.2 mol L^{-1}, the temperature of the column incubator was set at 40 °C, and the detector wavelength was set at a wavelength of 208 nm. Standard solutions in the concentration range from 10 to 200 mgL^{-1} were dissolved in acetonitrile and used for quantification purposes. Standard curves were constructed with concentration on the abscissa and peak area on the ordinate.

The glucoside alkaloids content (x) was calculated as c × v × f/m. Here, x—the content of the target in the sample, in grams (mg kg^{-1}); c—determination of target concentration in the sample solution from the calibration curve, in grams (mg kg^{-1}); v—the final constant volume of the sample solution to be measured, in grams (mL); m—the mass of the sample measured, in grams (g); f—diluted multiples.

2.5. Determination of Moisture Content and Weight Loss

2.5.1. Determination of Moisture Content

The direct drying method, as outlined in GB5009.3-2016 [16], was employed for assessing the moisture content in potato tubers during storage. The moisture content (x) was calculated as $(m_1 - m_2)/(m_1 - m_3) \times 100$. Here, x—the content of moisture in the sample, in grams (g/100 g); m_1—the mass of the weighing bottle (plus sea sand, glass rod) and the sample, in grams (g); m_2—the mass of the weighing bottle (plus sea sand and glass

rod) and the sample after drying, in grams (g); m_3—the mass of the weighing bottle (plus sea sand and glass rod), in grams (g); 100—unit conversion factor.

2.5.2. Determination of Weight Loss

The masses of each tuber before and after storage were individually determined. The rate of weight loss (x) was calculated as $(m_1 - m_2)/m_1 \times 100$. Here, x—weight loss rate in te sample, in grams (%); m_1—mass of the tuber before storage, in grams (g); m_2—mass of the tubers after storage, in grams (g); 100—unit conversion factor.

2.6. Determination of Browning

2.6.1. Determination of Browning Index

The browning index determination was carried out according to the method of Wang et al. [17] with minor modifications. The supernatant was obtained by collecting a 5 g sample of frozen and ground potato tubers. Subsequently, 3 mL of pre-cooled (4 °C) 95% ethanol solution was added, followed by incubation at 4 °C for 6 h and centrifugation at 12,000× *g* for 20 min. The absorbance at a wavelength of 410 nm was measured using the control group containing only 95% ethanol. The calculation formula used was $BD = \Delta OD_{410} \times 6$ (where ΔOD_{410} represents the absorbance value at a wavelength of 410 nm).

2.6.2. Determination of Total Phenolic Content

Folin phenol colorimetry was employed to determine the total phenolic content in extracts of potato samples as described by Joly et al. [18]. A frozen potato sample was pulverised into a fine powder using a grinder and liquid nitrogen. The following steps were followed: Take 3.0 g of the potato powder and grind it thoroughly in a mortar, then add 10 mL of methanol and refrigerate at 4 °C for 12 h. Subsequently, mix 300 µL of the extract with 1.7 mL of distilled water, followed by adding 1 mL each of Folin phenol solution and a 7.5% anhydrous sodium carbonate solution. The mixture was kept warm in a water bath at 25 °C for 2 h, after which the absorbance was measured at a wavelength of 765 nm. Notably, the standard curve equation is $y = 0.0111x + 0.0203$ with an R^2 value of 0.9954 (where x represents gallic acid concentration and y represents light absorption).

2.6.3. Determination of PPO Activity

PPO activity in potato tubers was determined using a plant PPO ELISA kit. After allowing the aluminium foil bag to equilibrate at room temperature for 20 min, we removed the required slats and sealed the remaining ones in a self-sealing bag before storing them at 4 °C. We set up standard product holes and sample holes, and added different concentrations of standard product (50 µL) to each standard hole. Then, we added 10 µL of the sample to the sample hole, followed by adding 40 µL of sample diluent. Blank holes were not added. In addition to blank holes, horseradish peroxidase (HRP)-labelled detection antibody (100 µL) was added to each well containing standards or samples, sealed with a sealing plate film, and incubated at 37 °C in a water bath or incubator for 1 h. Liquid contents were discarded and pat dry on absorbent paper. Then, each well was filled with washing solution for one minute before shaking off excess liquid and patting it dry on absorbent paper again, and this process was repeated five times. Substrates A and B (50 µL each) were added into every well and then incubated without light at 37 °C for 15 min. A termination solution (50 µL) was added into every well and then the OD value was measured within fifteen minutes using a wavelength of 450 nm. The standard curve equation is $y = 0.001x + 0.1139$ with an R^2 value of 0.9923 (where x represents PPO activity and y represents the OD value at a wavelength of 450 nm).

2.7. Determination of Respiration Intensity and MDA Content

2.7.1. Determination of Respiration Intensity

The respiration intensity of potato tubers was measured using a gas analyser, following the method of Yuan et al. [12]. The respiration intensity (Q) was calculated as $(V \times N \times 1.894 \times 1000)/(m \times t)$, where Q is the respiration intensity [mg/(kg·h)]; V is the volume of the container (plastic basket volume with potato volume, L); N is the percentage of CO_2 (%); 1.894 is the density of carbon dioxide at 10 °C under normal pressure; m is the mass of the sample (kg); and t is the airtight time (h).

2.7.2. Determination of MDA Content

The MDA content of the samples was determined following the method of Palamutoğlu et al. [19] with minor modifications. The potato sample (1 g) was homogenised with 10 mL of pre-cooled trichloroacetic acid solution (1 g/L) and centrifuged at 4000 rpm for 15 min at 4 °C. The resulting supernatants were used for MDA analysis. A mixture of 0.6 mL supernatant and 1 mL thiobarbituric acid solution (6.7 g/L) was prepared in glass tubes and incubated at a temperature of 95 °C for a duration of 20 min. Subsequently, the mixture was cooled using running water, followed by measuring its absorbance at wavelengths of 450 nm, 532 nm, and 600 nm. Finally, equation (6) was employed to calculate the MDA content (C), which was calculated as $6.45 \times (OD_{532} - OD_{600}) - 0.56 \times OD_{450}$. In the formula, OD_{450}, OD_{532}, and OD_{600} represent the absorbance at 450, 532, and 600 nm, respectively. C denotes the concentration of MDA in μmol/kg.

2.8. Determination of Reducing Sugar and Starch Content

2.8.1. Determination of Reducing Sugar Content

The concentration of reducing sugar was determined using the 3,5-dinitrosalicylic acid method as described by Hu et al. [20]. A total of 1.0 g of potato powder was weighed, washed, and placed into a conical flask with 50 mL of distilled water. After thorough mixing, the mixture was heated in a water bath at 50 °C for 30 min. Once cooled, the homogenate was filtered, and the filtrate was transferred to a 100 mL volumetric flask for volume adjustment. The absorbance of the resulting solution was measured at a wavelength of 540 nm. The standard curve equation is y = 0.9817x − 0.0412 with an R^2 value of 0.9962 (where x represents the quality of sample and y represents the OD value at a wavelength of 540 nm). The reducing sugar content was calculated as Reducing sugar content $= \frac{m' \times V \times N}{V_s \times m}$. Here, m'—mass of glucose in the standard curve, in grams (μg); V—mass of total volume of extract, in grams (mL); N—mass of sample dilution fold; Vs—mass of the volume of sample aspirated during determination, in grams (mL); m—mass of the sample, in grams (g).

2.8.2. Determination of Starch Content

For the determination of starch content, the iodine-starch colorimetric method [12] was followed. The starch content was calculated using equation Starch content/% $= \frac{m' \times V \times N}{Vs \times m \times 10^6}$. Here, m' is the mass of starch in the standard curve (μg); V is the total volume of extract (mL); N is the sample dilution fold; Vs is the volume of sample aspirated during determination (mL); and m is the sample mass (g).

2.9. Determination of Soluble Protein and Amino Acid Content

2.9.1. Determination of Soluble Protein Content

The soluble protein content in potatoes was determined following the method of Wegener et al. [21]. The calculation was performed using an equation of soluble protein content = $\frac{m' \times V}{Vs \times m \times 1000}$. Here, m' is the mass of soluble protein in the standard curve (μg); V is the total volume of the extract (mL); Vs is the volume of the sample aspirated during determination (mL); and m is the sample mass (g).

2.9.2. Determination of Amino Acid Content

The amino acid content was determined using an amino acid analyser according to the method outlined in GB 5009.124-2016 [22] in China.

2.10. Data Analysis

Three parallel trials were conducted for each treatment group, and the significance of differences in the test results was analysed using SPSS 23.0 software. After one-way analysis of variance, Duncan's multiple comparisons were employed to analyse differences between the data at a significance level of $p < 0.05$. Graphs were generated using Excel 2010 software, and the results were expressed as mean ± standard deviation.

3. Results and Analysis

3.1. Effect of Menthol Treatment on Potato Tuber Sprouting

During the storage period, daily observations of potato tuber sprouting were made, and, on the 15th d, a morphological picture of potato sprouting was taken. As depicted in Figure 1A, both the CK group and the 0.2 g/kg group exhibited sprouting, with budding observed on the lateral side of the tuber. The buds appeared yellow, while the apical buds were black. In contrast, the 0.5 g/kg group showed no signs of sprouting. The tuber eyes were completely black, the apical bud did not sprout, and other parts of the eyes were necrotic. This suggests that menthol treatment can damage the meristematic tissues of potato tubers, causing injury to the apical meristems, resulting in skin damage and bud necrosis, ultimately inhibiting potato sprouting.

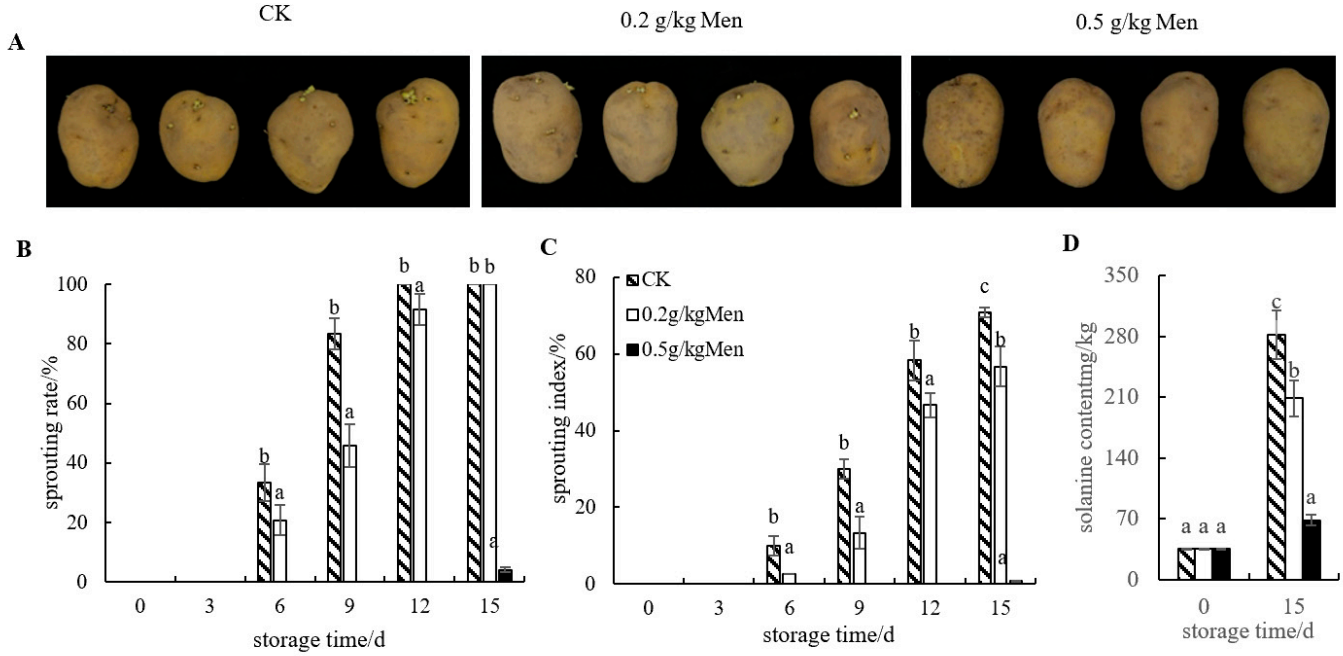


Figure 1. Impact of menthol treatment on the sprouting state (**A**), sprouting rate (**B**), sprouting index (**C**), and solanine content (**D**) in potato (*Solanum tuberosum* L.) tubers. Different letters on the same day indicate a significant ($p < 0.05$) difference. The data represent mean ± SD of three replicates.

The impact of menthol on potato sprouting percentage and sprouting index is illustrated in Figure 1B,C, respectively. In Figure 1B, it is evident that from day 0 to day 3, no sprouting occurred in any of the three treated groups. By day 6, both the control group (CK) and the 0.2 g/kg group initiated sprouting, with sprouting percentages of 33.3% and 20.8%, respectively, while the 0.5 g/kg group remained free from sprouting. On day 12, the sprouting percentages for CK and the 0.2 g/kg group were 100% and 91.67%, respectively, while the 0.5 g/kg group continued to show no signs of sprouting. After 15 days of storage,

the sprouting percentage for the 0.5 g/kg group was 4.1%, significantly lower than that of CK and the 0.2 g/kg group ($p < 0.05$).

Turning to Figure 1C, it is observed that starting from day 6, the sprouting index of the CK group and the 0.2 g/kg group exhibited an increasing trend. Moreover, the sprouting index of the CK group was significantly higher than that of the 0.2 g/kg group ($p < 0.05$). At the conclusion of storage, the sprouting index for CK, 0.2 g/kg, and 0.5 g/kg groups was 70.8%, 56.6%, and 0.8%, respectively, indicating significant differences ($p < 0.05$) in the sprouting index among these three groups. These results affirm that menthol effectively inhibits potato tuber sprouting.

3.2. Impact of Menthol Treatment on Glucoside Alkaloid Content in Potatoes

Following storage, potatoes may exhibit elevated glucoside alkaloids levels due to tuber sprouting, particularly in the sprouting and greening sections of the potato. Figure 1D illustrates the effect of menthol on glucoside alkaloid content in potatoes after storage. After 15 days of storage, the glucoside alkaloid content in the 0.5 g/kg group was 68.63 mg/kg, significantly lower than that in the CK group (282.01 mg/kg) and the 0.2 g/kg group (208.91 mg/kg) ($p < 0.05$). The Chinese national limit for glucoside alkaloids is 200 mg/kg, confirming that the glucoside alkaloid content in the 0.5 g/kg group complies with national safety standards. A comparative analysis across different storage times and treatment groups suggests that high concentrations of menthol effectively inhibit glucoside alkaloid accumulation in potatoes by suppressing sprouting.

3.3. Impact of Menthol Treatment on Potato Moisture Content, Weight Loss, Respiration Intensity, and MDA Content

Figure 2A,B showcase the effect of menthol on the moisture content and weight loss of potatoes during storage. As observed in Figure 2A, the moisture content of potatoes significantly increased during the initial six days of storage. Subsequently, after the sixth day, the moisture content exhibited a gradual decline. This phenomenon can be attributed to the initial storage of potatoes at 4 °C. The influence of enzymes, such as ADP-glucose pyrophosphorylase, UDP-glucose pyrophosphorylase, and sucrose synthase, leads to starch conversion and breakdown into reducing sugars [23]. The accumulation of these reducing sugars results in low-temperature sweetening. During this process, the free water content becomes lower than the bound water content, contributing to an overall lower moisture content [24]. Conversely, in the early stages of potato storage, as the temperature rises, previously accumulated reducing sugars revert to starch, causing the free water content to exceed the bound water content, resulting in a higher moisture content. Comparative analysis among experimental groups indicates that the moisture content of the 0.5 g/kg group surpassed that of the 0.2 g/kg group and the CK group during storage, indicating that a high menthol concentration effectively reduces water loss and maintains higher moisture content.

In Figure 2B, the weight loss rate of potatoes gradually increased during storage. The CK group exhibited a significantly higher weight loss rate than the 0.2 g/kg and 0.5 g/kg groups ($p < 0.05$). At the end of the storage period, the weight loss rates were 0.83% in the CK group, 0.74% in the 0.2 g/kg group, and 0.67% in the 0.5 g/kg group ($p < 0.05$). This indicates that menthol-treated potatoes effectively inhibit respiration, transpiration, and other processes, thereby reducing nutrient losses.

Respiration intensity reflects the strength of plant metabolism, as depicted in Figure 3A, showcasing changes in potato respiration intensity during storage. The three groups exhibited an initial increase followed by a decrease in respiration intensity throughout the storage period, with the most pronounced trend observed in the 0.2 g/kg group. Comparing different experimental groups, on day 3 of storage, the respiration intensity of the 0.5 g/kg group (9.78 mg/(kg·h)) was significantly higher than that of the CK group (4.38 mg/(kg·h)) and the 0.2 g/kg group (7.10 mg/(kg·h)) ($p < 0.05$). This may be attributed to the high menthol concentration triggering a stress response in the potatoes. On day

15 of storage, the respiration intensity in the 0.5 g/kg group (3.13 mg/(kg·h)) was significantly lower ($p < 0.05$) than that in the CK group (3.70 mg/(kg·h)) and the 0.2 g/kg group (4.11 mg/(kg·h)). This suggests that menthol-treated potatoes displayed robust early-stage metabolic activity and maintained a higher respiration intensity. In the middle and later stages, the treated potatoes exhibited a relatively stable metabolic state, indicating that menthol treatment can inhibit potato respiration intensity. This aligns with the results of sprouting percentage and sprouting index, indicating increased respiration intensity during potato tuber sprouting in the CK group.

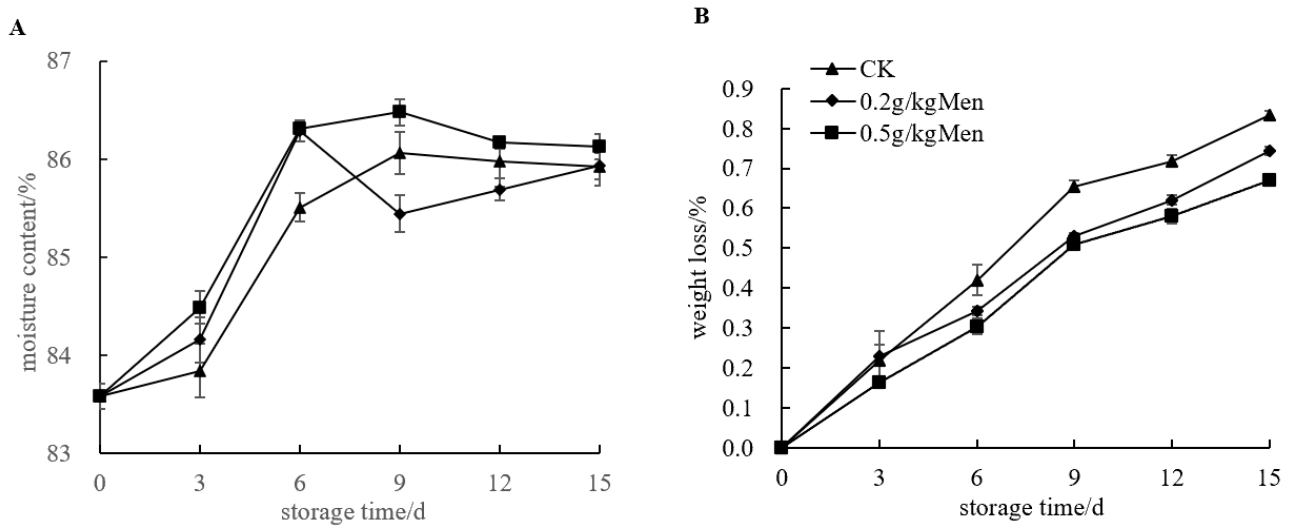


Figure 2. Impact of menthol treatment on the moisture content (**A**) and weight loss (**B**) in potato (*Solanum tuberosum* L.) tubers.

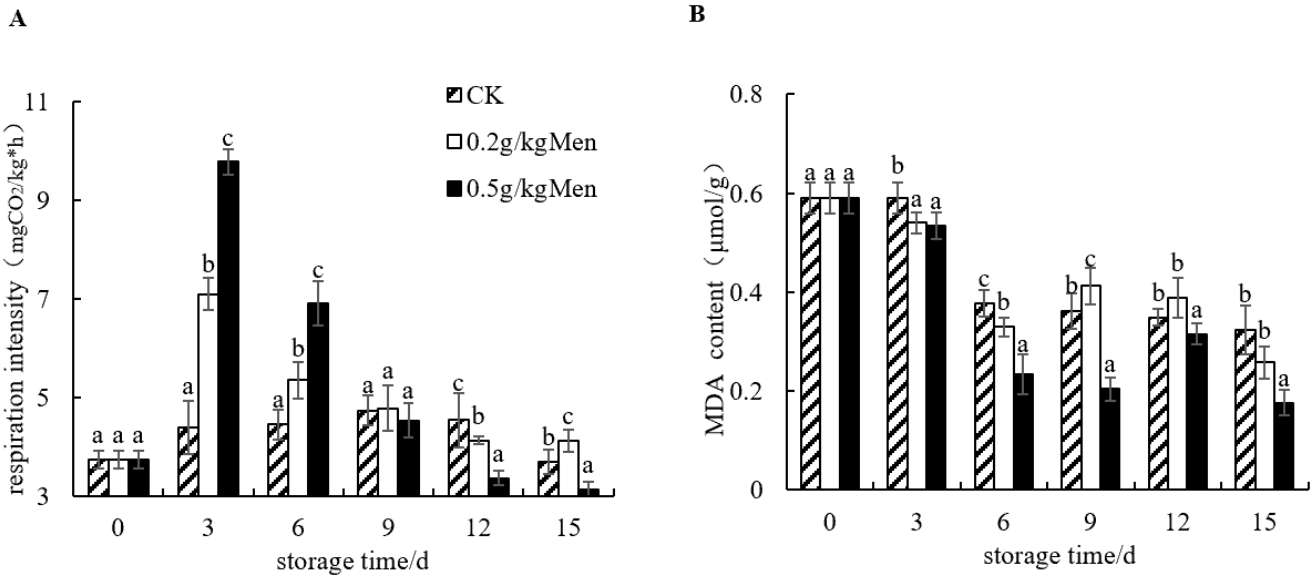


Figure 3. Impact of menthol treatment on the respiration intensity (**A**) and MDA (malondialdehyde) content (**B**) in potato (*Solanum tuberosum* L.) tubers. Different letters indicate a significant ($p < 0.05$) difference. Data are presented as the mean ± SD of three replicates.

MDA is a major byproduct in the process of cell membrane lipid peroxidation, with a high concentration having a detrimental impact on the cell membrane [25]. MDA content serves as an indicator of membrane lipid peroxidation, reflecting damage to the membrane system and plant stress tolerance [26]. Figure 3B depicts the fluctuations in potato MDA content during storage. The MDA content across the three groups exhibited a general decreasing trend throughout storage. A comparative analysis indicated that the 0.5 g/kg

group consistently maintained a lower MDA content than the CK and 0.2 g/kg groups. At the end of the storage period (15 days), the MDA content was 0.18 μmol/g in the 0.5 g/kg group, significantly lower than that in the CK group (0.32 μmol/g) and the 0.2 g/kg group (0.26 μmol/g) ($p < 0.05$). This underscores the substantial inhibitory effect of high menthol concentration on the accumulation of harmful substances in potatoes.

3.4. Impact of Menthol Treatment on Browning Index, Total Phenol Content, and PPO Activity in Potatoes

The browning index serves as an indirect gauge of soluble pigments generated in the browning reaction, reflecting the degree of browning in fruits and vegetables [27]. Figure 4A illustrates the changes in the browning index among the three potato groups during storage. Over time, the browning index in all groups exhibited an ascending and then descending trend. A comparative analysis indicated that the browning index of the 0.5 g/kg group remained consistently lower than that of the CK and 0.2 g/kg groups throughout the storage period. At the conclusion of the storage period (15 days), the browning index reached 0.42 in the 0.5 g/kg group, significantly lower ($p < 0.05$) than the values of 0.54 and 0.51 in the CK and 0.2 g/kg groups, respectively. This underscores the effective ability of 0.5 g/kg menthol to delay and inhibit potato browning, preserving the quality of potatoes and achieving a preservation effect.

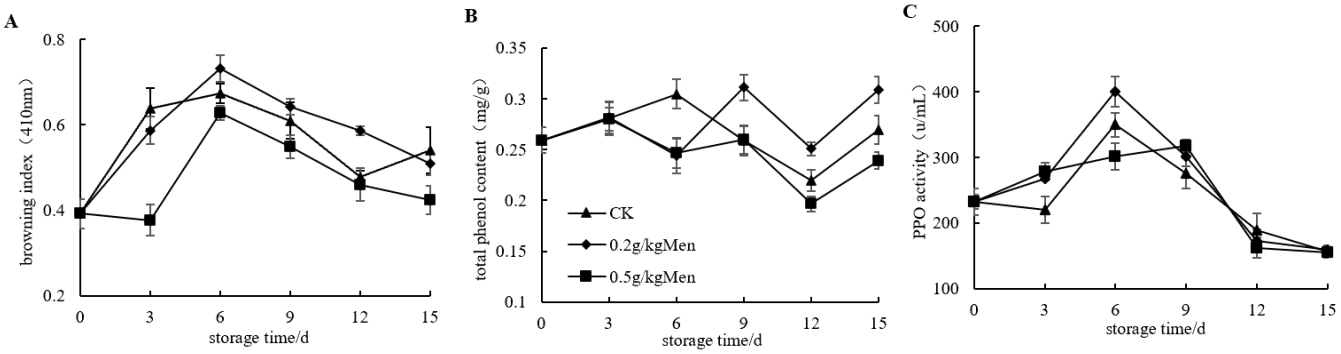


Figure 4. Impact of menthol treatment on the browning index (**A**), total phenol content (**B**), and PPO (polyphenol oxidase) activity (**C**) in potato (*Solanum tuberosum* L.) tubers.

Plants harbour a diverse array of phenolics, crucial substrates for the enzymatic colouring of fruits and vegetables [28]. In Figure 4B, the total phenol content in potato tubers exhibits erratic variations across all groups during the storage period. There was a declining trend in total phenol content from day 3 to day 6, suggesting phenolics were utilised by phenolases, contributing to enzymic browning and accelerating the tuber browning rate, consistent with the observed changes in browning degree. In the later stages, as the browning rate slowed, total phenol accumulation increased, resulting in a gradual rise in total phenol content after 12 days. Upon the comparison of experimental groups, the total phenol content of the 0.5 g/kg group consistently lagged behind that of the CK and 0.2 g/kg groups. At the end of storage, the total phenol content in the 0.5 g/kg group was 0.23 mg/g, significantly lower than the CK group (0.27 mg/g) and the 0.2 g/kg group (0.31 mg/g) ($p < 0.05$). This indicates that menthol-treated potato tubers experienced a gradual change in total phenol content, exhibiting a slow browning rate and maintaining good storage quality.

PPO serves as the primary enzyme responsible for the enzymatic browning of potatoes, impacting their flavour and quality [29,30]. PPO activity is a crucial indicator for assessing the extent of browning. Additionally, PPO functions as a terminal oxidase system in plant tissue respiration, influencing dormancy and sprouting in tubers [31]. Within 15 days of storage, the binding of PPO to the inner vesicle membrane may be compromised, leading to PPO activation [32]. As depicted in Figure 4C, PPO activity exhibited an initial increase (during the first six days) followed by a decrease during storage across all groups. A

comparative analysis among the three groups on day 6 revealed that the PPO activity of the 0.5 g/kg group was 301.43 U/mL, significantly lower than that of the CK group (400.10 U/mL) and the 0.2 g/kg group (349.77 U/mL) ($p < 0.05$). Even at the end of the storage period, the PPO activity of the 0.2 g/kg group remained significantly lower than that of the CK group and the 0.2 g/kg group ($p < 0.05$). In conclusion, menthol treatment demonstrated effective inhibition of PPO activity, showcasing a positive correlation with the observed changes in browning degree in this study.

3.5. Effect of Menthol Treatment on Reducing Sugar and Starch Content of Potatoes

In Figure 5A, it is observed that during the initial storage phase, the potato exhibited the highest reducing sugar content of 173.47 mg/g. This could be attributed to the storage at 4 °C before testing, where the activity of the amylolytic enzyme in the tuber surpassed that of amylopectin synthase, resulting in the conversion of starch to reducing sugar [33]. In the early storage period (up to day 6), the reducing sugar content in all groups declined due to increased respiration and the conversion of reducing sugars into starch after removal from cold storage. However, a temporary surge in reducing sugar content occurred during days 6~12, followed by a subsequent decline from days 12~15. Comparative analysis at the late storage stage revealed that the reducing sugar content of the 0.5 g/kg group was lower than that of both the CK and 0.2 g/kg groups. At the end of storage, the reducing sugar content of the 0.5 g/kg group was 80.30 mg/g, significantly lower than that of both the CK group (121.86 mg/g) and the 0.2 g/kg group (99.58 mg/g) ($p < 0.05$). These findings indicate that menthol markedly inhibited the accumulation of reducing sugars during the storage of potato tubers.

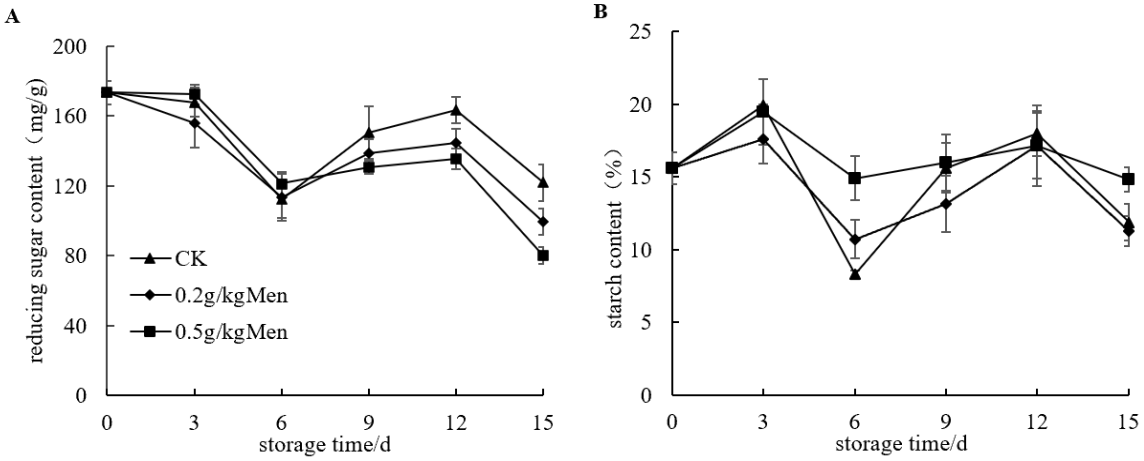


Figure 5. Impact of menthol treatment on the reducing sugar content (**A**) and starch content (**B**) in potato (*Solanum tuberosum* L.) tubers.

Starch serves as the primary nutrient and energy source in potato tubers, reflecting changes in storage quality [34]. In Figure 5B, the starch content initially rises, falls, then rises again with prolonged storage. Comparisons reveal that the 0.5 g/kg group consistently maintains a higher starch content than the CK group. At the 6th day, the 0.5 g/kg group reaches 14.92%, significantly surpassing the CK group (8.33%) and the 0.2 g/kg group (10.70%) ($p < 0.05$). At the storage end, the starch content for the 0.5 g/kg, 0.2 g/kg, and CK groups is 14.84%, 10.86%, and 21.89%, respectively, compared to their initial values of 95.18%, 72.21%, and 76.27%. In summary, high-concentration menthol treatment effectively retards starch content decline, favouring quality maintenance during storage.

3.6. Effect of Menthol Treatment on Soluble Protein and Amino Acid Content of Potatoes

Soluble proteins serve as the foundation for physiological metabolism in fruit and vegetable cells, with intricate enzyme systems primarily comprising non-membrane-bound proteins [21]. Table 2 illustrates the impact of menthol on soluble protein content in potato

tubers. Throughout storage, soluble protein content steadily increased. The CK group consistently exhibited lower soluble protein content, possibly due to untreated potato sprouting, leading to higher metabolic activity and energy consumption. At the 12th and 15th days of storage, the 0.5 g/kg group showed significantly higher soluble protein content (4.06 mg/g) than the CK and 0.2 g/kg groups during the same period ($p < 0.05$), indicating menthol's effective delay in soluble protein loss, showcasing superior preservation.

Table 2. Impact of menthol treatment on the soluble protein content in potato tubers.

Soluble Protein Content (mg/g)	Treatment Groups		
	CK	**0.2 g/kg Men**	**0.5 g/kg Men**
0 d	3.18 ± 0.07 [a]	3.18 ± 0.07 [a]	3.18 ± 0.07 [a]
3 d	3.21 ± 0.05 [a]	3.62 ± 0.05 [b]	3.69 ± 0.04 [b]
6 d	2.99 ± 0.12 [a]	3.38 ± 0.11 [b]	3.43 ± 0.11 [b]
9 d	3.55 ± 0.17 [a]	3.78 ± 0.09 [a]	3.63 ± 0.10 [a]
12 d	3.73 ± 0.07 [a]	3.71 ± 0.03 [a]	4.06 ± 0.08 [b]
15 d	3.87 ± 0.05 [a]	3.86 ± 0.06 [a]	4.06 ± 0.08 [b]

The different letters indicate a significant ($p < 0.05$) difference. Data are presented as the mean ± SD of three replicates.

Amino acids, crucial osmotic regulators, can reduce potato browning by forming colourless adducts with quinones or acting as chelating agents [10]. Table 3 displays the menthol effect on amino acid content in potatoes before and after storage. At the storage end, the 0.5 g/kg group exhibited lower glycine, methionine, and proline content (0.39 mg/g, 0.021 mg/g, and 0.43 mg/g, respectively) compared to the 0.2 g/kg group and the CK group. This may be attributed to untreated and 0.2 g/kg menthol treatment group containing higher glycine, methionine, and proline, forming coloured catechol-amino acid adducts and inducing browning [35]. However, further exploration is needed for a comprehensive understanding of this mechanism. The majority of amino acid contents in potatoes remained stable during storage, suggesting minimal impact on amino acid content due to menthol.

Table 3. Impact of menthol treatment on the amino acid content in potato tubers.

Amino Acid Content (mg/g)	0 d	15 d		
	Control Value	**CK**	**0.2 g/kg Men**	**0.5 g/kg Men**
glycine	0.43 ± 0.015	0.43 ± 0.017 [a]	0.43 ± 0.020 [a]	0.39 ± 0.016 [a]
methionine	0.03 ± 0.0025	0.026 ± 0.0021 [b]	0.025 ± 0.0025 [b]	0.021 ± 0.018 [a]
proline	0.39 ± 0.011	0.49 ± 0.031 [b]	0.45 ± 0.034 [a]	0.43 ± 0.027 [a]
total amino acid	14.67 ± 0.105	14.256 ± 0.162 [b]	14.005 ± 0.128 [a]	13.891 ± 0.131 [a]

The different letters indicate a significant ($p < 0.05$) difference. Data are presented as the mean ± SD of three replicates.

3.7. Correlation Analysis of Potato Germination and Quality Indicators

To comprehensively understand the correlation between germination and quality indicators during the storage process of potatoes, a correlation analysis was conducted on relevant indicators of potatoes under different experimental groups and storage times (Figure 6). The results revealed varying degrees of correlation between the indicators during potato storage. Notably, there is an extremely significant positive correlation coefficient of 0.962 ($p < 0.01$) between the sprouting rate and sprouting index, indicating high data accuracy in this research. Additionally, both sprouting rate and sprouting index exhibit highly significant positive correlations with the rate of weight loss ($p < 0.01$), yielding respective correlation coefficients of 0.750 and 0.728. Furthermore, they also show significant positive correlations with moisture content ($p < 0.05$), with respective correlation coefficients of 0.541 and 0.486. The weight loss rate exhibits an extremely significant positive correlation with moisture content ($p < 0.01$), displaying a correlation coefficient of 0.832. This strong association can be attributed to the dynamic interplay between free water and

bound water induced by low-temperature glycosylation during potato storage, resulting in an upward trajectory of potato moisture content throughout the storage period. The moisture content exhibits a highly significant negative correlation with both MDA content and reducing sugar content ($p < 0.01$), with correlation coefficients of −0.912 and −0.750, respectively. The MDA content has a remarkably significant positive correlation with reducing sugar content ($p < 0.01$), with a correlation coefficient of 0.841. The reducing sugar content has a remarkably significant positive correlation with starch content ($p < 0.01$), with a correlation coefficient of 0.655. The browning index has a significant positive correlation with PPO activity ($p < 0.05$), with a correlation coefficient of 0.554, consistent with the results of this research.

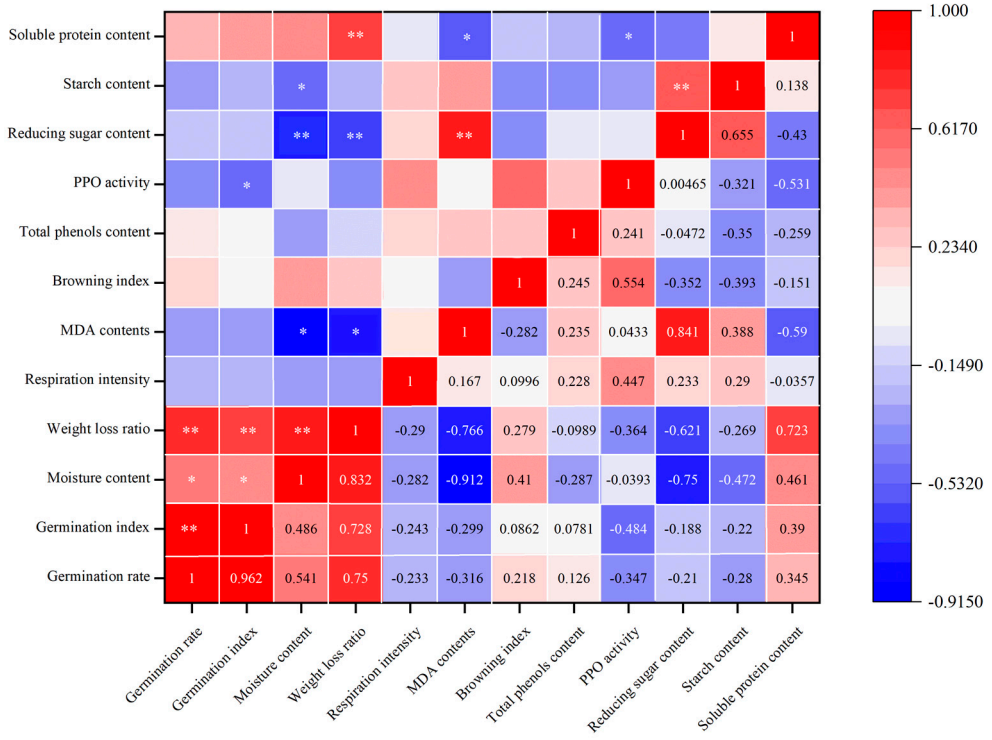


Figure 6. Heat map analysis of correlation coefficient of germination and quality indicators of potatoes during storage. Red (+1) indicates a positive correlation between different indicators, while blue (−1) indicates a negative correlation. ** indicates a significant correlation at the 0.01 level (bilateral); * indicates a significant association at the 0.05 level (bilateral).

4. Conclusions

This study investigated the impact of menthol on sprout inhibition and storage quality in potato tubers, utilising two menthol concentrations. The findings indicate that the postharvest application of 0.5 g/kg menthol effectively hinders tuber sprouting, leading to a decreased sprouting percentage and sprouting index, along with reduced glucoside alkaloid production. Notably, 0.5 g/kg menthol treatment induced the blackening of potato tuber buds and disrupted bud tissue during sprouting, resulting in delayed sprouting.

In addition, the research also observed that menthol treatment maintained higher respiration levels in potato tubers during the early storage stage, which declined after wound healing, and suppressed potato respiration intensity during the late storage stage. PPO activity and the total phenolic content in potatoes correlate with browning. The results demonstrated that 0.5 g/kg menthol concentrations significantly reduced PPO activity and total phenol content at the end of storage compared to control and 0.2 g/kg menthol concentration groups, positively correlating with the browning degree. The 0.5 g/kg menthol treatment delayed the decline in potato starch content, inhibited reducing sugar production, and maintained low reducing sugar levels. After breaking dormancy, proteins

broke down into amino acids to support bud development. Soluble protein content in the 0.5 g/kg menthol treatment group remained higher than the control and 0.2 g/kg menthol treatment at the end of storage, suggesting that menthol slows soluble protein depletion and delays potato aging, with less obvious effects on amino acid content.

In conclusion, menthol, a natural plant ingredient, not only exhibits a significant sprout inhibition effect but also enhances the overall quality of potatoes during storage, preserving their commercial value. Moreover, menthol significantly affects the colour quality and carbohydrate content during potato sprouting, with a relatively minor influence on protein metabolism. Although the glucoside alkaloids content in the 0.5 g/kg menthol treatment group was found to be below the national safety standard, its presence remained detectable. Further investigation is needed to unveil the specific physiological, biochemical, and molecular mechanisms underlying these observed effects.

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