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Controlled atmosphere as cold chain support for extending postharvest life in cabbage

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ABSTRACT

Postharvest management of cabbage relies on high-intensity cooling to control postharvest physiology, minimising quality loss despite incurring significant energy and environmental costs. As an alternative, we hypothesised that controlled atmosphere (CA) could allow increased storage temperature by supporting physiological regulation, while maintaining quality and reducing energy demand. This study examined the effect CA (1.5 kPa CO_2 and 6 kPa O_2) at 5 or 10 °C on cabbage quality, with the aim of proposing a more sustainable and resilient supply chain. CA treatment was effective at reducing head respiration at higher temperature, with CA/10 °C treatment achieving lower respiration rates than Control/5 °C. Improved head colour retention and maintenance of stump quality were observed in cabbage under CA conditions. CA effects were seen also at a regulatory level; CA promoted an average of 25.4% reduction in abscisic acid accumulation potentially as part of a wider hypoxia stress response and was successful in decreasing expression of the senescence-coordinating transcription factor *BoORE15*. This finding was linked with a lower in downstream expression of pheophytinase and subtilisin protease. These results demonstrated that CA treatment fundamentally modified postharvest physiology in cabbage, which can be exploited to enable storage at warmer temperatures, contributing to supply chains with lower energy demand and its associated environmental benefits.

1. Introduction

Cabbage (*Brassica oleracea* var. *capitata*) is a major vegetable crop, with 186 kT consumed in the United Kingdom (UK) annually (Department of Food and Rural Affairs, 2022). Year-round demand is met by field and/or cold storage (0–1 °C) combined with intermediate storage at 5 °C in the supply chain, although postharvest losses can exceed 20% due to senescence and disease (Gage et al., 2024). Cabbage is valued for its abundant health-promoting compounds such as carotenoids and glucosinolates, but these decline during storage, incurring nutritional losses (Hu et al., 2021; Casajús et al., 2023). Outer leaf senescence gives rise to discolouration, textural changes, deterioration of stump condition and disease activity which reduces marketable volume, either through whole-head loss or increased trimming requirements. Food loss reduction strategies rely on the application of postharvest solutions that slow down developmental and senescence processes, as well as inhibiting microbial activity which contribute to decay (Falagán and Terry, 2018). However, postharvest management creates significant environmental and economic costs associated with the high energy demand from cooling, with one third of the total greenhouse emissions from cabbage produced during storage (Frankowska et al., 2019). Furthermore, low economic value and varied storage practice (e.g. cold storage, field overwintering) has hindered the development of postharvest approaches for cabbage relative to other brassicas (e.g. broccoli). To address this challenge, we sought to develop alternative postharvest technologies to improve the sustainability of the cabbage supply chain by allowing the increased storage temperature while maintaining physiological and nutritional quality.

Slowing down the postharvest metabolism of fresh produce through manipulation of the gaseous environment (controlled atmosphere [CA]) is well documented and has been shown to be effective in reducing loss of colour (chlorophyll), turgor and tissue integrity in broccoli during cold storage (Eason et al., 2007; Schouten et al., 2009; Büchert et al., 2011). CA treatment triggers regulatory changes, many of which are aligned with hypoxic stress responses (Cukrov et al., 2016). CA is also likely to interact with multiple signalling pathways that coordinate senescence, with reductions in abscisic acid (ABA) and ethylene precursors, as seen in Chinese cabbage (*B. campestris* var. pekinensis) under

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CA (Wang and Ji, 1988). The use of CA to regulate postharvest metabolism as a low-energy complement to cooling has been previously recognised (East et al., 2013). However, its application to cabbage is hindered by a lack of evidence regarding optimal CA conditions for this crop. The physiological basis of CA treatment is poorly understood, and responses vary with storage temperature, cultivar, and physiological status (Schouten et al., 2009; Büchert et al., 2011; Huang et al., 2020), making it challenging to predict the optimal storage conditions for cabbage. In order to examine the potential for CA to maintain cabbage quality under increased storage temperatures, this study aimed to elucidate the relationship between temperature, gaseous environment, and postharvest response in cabbage. Modulation of senescent activity was examined through profiling of plant growth regulatory changes and using senescent bioindicators at a gene regulatory level.

2. Materials and methods

2.1. Plant material and postharvest treatments

Trimmed green cabbage (*Brassica oleracea* var. *capitata* "Zenon Epikur") samples were obtained from a commercial grower on the February 15, 2023 within 6 h of harvest, and selected for uniformity of size (2–3 kg) and freedom from damage/disease. Heads were stored at two different temperatures (5 °C and 10 °C) at 95% humidity for 28 days. Two gaseous treatments were applied at both temperatures: i) Control (air; 0.03 kPa carbon dioxide [CO_2] and 20.9 kPa oxygen [O_2]); and ii) CA (1.5 kPa CO_2 and 6 kPa O_2), which were identified as being efficacious for brassica previously (e.g. Menniti et al., 1997; Nakamura et al., 2013). Gas composition was supplied using mass flow controllers (Alicat Scientific, USA). Heads destined for sampling were stored in 100 L polypropylene boxes (Chiltern Industrial Services Ltd., Bedford, UK) under 0.8 L min^{-1} airflow which were water-sealed except for sampling. Heads for real-time respiration measurements were stored in 16 L airtight polypropylene boxes with an airflow of 0.4 L min^{-1} which were sealed for the duration of the experiment. Gas treatments were periodically verified through head space gas analysis (GS3/P Analyzer, Systech, Illinois, USA), and airflow rates were set to ensure consistent atmospheric composition irrespective of head gas exchange rates through frequent atmosphere replacement.

Samples were collected on days 0, 4, 7, 14, 21 and 28, with replicates consisting of two heads sampled in triplicate, totalling six baselines heads and 30 heads per treatment. At sampling, fresh heads were imaged using a Canon EOS 90D (32 MP) camera coupled with EF-S 18–55 mm f/3.5–5.6 IS STM lenses (Canon, UK) under constant lighting using a photobox equipped with 5500K LED lamps. Following imaging, 3 cm radial wedges were cut from each head for biochemical analysis, and a 1 cm leaf disc was cut from the outer most leaf for RNA extraction. Samples were snap frozen in liquid nitrogen prior to storage at −80 °C. Before biochemical analysis, samples were freeze dried at −50 °C (Scanvac CoolSafe Pro, Labogene Ltd., Denmark) before powdering and storage at −40 °C.

2.2. Real-time respiration rates

Continuous real-time respiration rate was measured using a Sable Respirometry System (model 1.3.8 Pro, Sable Systems, USA) and reported as mmol of CO_2 produced per kg and hour (Alamar et al., 2017). Measurements were taken from three replicate boxes (each containing one head), including an additional empty baseline box utilised to prevent cross contamination between recording events. Automated switching was achieved with a SS4-subsampler enabled recording of flow rate, water vapour pressure, O_2 and CO_2 concentrations using a RH-300, CA-10 and FC-10 system respectively (all Sable Systems, USA). Changes in O_2 and CO_2 concentrations in gas feeds were recorded for 120 s in a cyclical fashion, interspersed with measurement of baseline boxes for 180 s to allow equilibration between samples.

2.3. Head quality

RGB values were determined for three 3.5 cm diameter circles per head from images taken prior to sampling (Fig. 1A), and for an elliptical selection fitted to the entire stump (Fig. 1B) using ImageJ (Schneider et al., 2012). Following conversion to CIELab colour space, Hue angle (°h = arctg (b^*/a^*) and Chroma ($C^* = (a^{*2}+b^{*2})^{1/2}$) were calculated following Voss (1992). Heads were scored for the presence/absence of adventitious rooting, and the length of nine roots >1 mm were recorded per head if present. Three representative heads per treatment were weighed at each sampling point to assess weight loss during storage (3000 Series balance, Ohaus Ltd., UK).

2.4. Biochemical analysis

2.4.1. Pigments

Photosynthetic pigments were quantified after García-Plazaola and Becerril (1999). Freeze-dried powdered samples (150 mg) were extracted using 1.5 mL 99% acetone with 1 mM ascorbic acid in darkness. Analysis was conducted using Ultra High Performance Liquid Chromatography (UHPLC) Agilent 1260 Infinity II Series, fitted with diode array detector (DAD) G7115A, pump G7104C, autosampler G7129C and column oven G7115. Detection was carried out at 445 nm, except for chlorophyll *a* at 410 nm. The detector was set at 410 nm for chlorophyll *a* and 445 nm for chlorophyll *b*. Separation was achieved using a Agilent Poroshell 120 column, 2.7 μm, 3 × 100 mm using two mobile phase solvents: acetonitrile:methanol:water (A – 84:9:7) and methanol:ethyl acetate (B – 68:32). Elution was achieved using a linear 100% A/B gradient for 4 min, followed by isocratic elution with 100% B for 2 min, and going back to initial conditions for 4 min. Pigments were quantified using external standards (Sigma-Aldrich, UK).

2.4.2. Organic acids

Citric, malic, and ascorbic acids were quantified following modifications of Crespo et al. (2010). Freeze-dried powdered samples (150 mg) were extracted using 3% HPO_3 (w/v) for 10 min on ice before filtration (0.2 μm polypropylene filters, Millipore). Analysis was conducted by HPLC fitted with a DAD (Agilent Technologies 1200 Series, G1315B) set at 210 nm. Separation was achieved using a Phenomenex Luna column, 4.5 mm × 250 mm, using 25 mM KH_2PO_4 adjusted to pH 2.5 with a HPO_3 mobile phase. Extracts were quantified using external standards (Sigma-Aldrich, UK).

2.4.3. Non-structural carbohydrates

Sucrose, glucose, and fructose were quantified following a modification of O'Donoghue et al. (2004). Freeze dried powdered samples (150 mg) were extracted using 62.5% (v/v) methanol at 55 °C for 15 min prior to filtration (0.2 μm polypropylene filters, Millipore). Extracts were analysed via HPLC (Agilent Technologies 1200 series, UK) using refractive index detector (RID, Agilent Technologies 1200 series, G1362A) and a Concise CarboSep 87C CHO FA column 100 × 7.8 mm with guard column. Ultra-pure water was used as mobile phase. Isocratic flow was set up for 0.6 mL min^{-1}, and the column was kept at 80 °C. Glucose, sucrose and fructose were quantified using external standards (Sigma-Aldrich, UK).

2.4.4. Plant growth regulators

Plant growth regulators (PGRs) were analysed according to Anastasiadi et al. (2020) with modifications. Concentrations of ABA and ABA glucose ester (ABA-GE) were quantified together with catabolites 7-OH-ABA, phaseic acid (PA), dihydrophaseic acid (DPA), and neophaseic acid (neoPA). For the cytokinin pathway, isopentenyl adenosine riboside (IPR), IPR phosphate (IPRP) and *cis*-zeatin (cisZ) were quantified. Freeze dried powdered samples (15 mg) were extracted with methanol:formic acid:water (60:5:35) including 5 ng mL^{-1} deuterated internal standards and 250 mg L^{-1} butylated hydroxytoluene. Samples

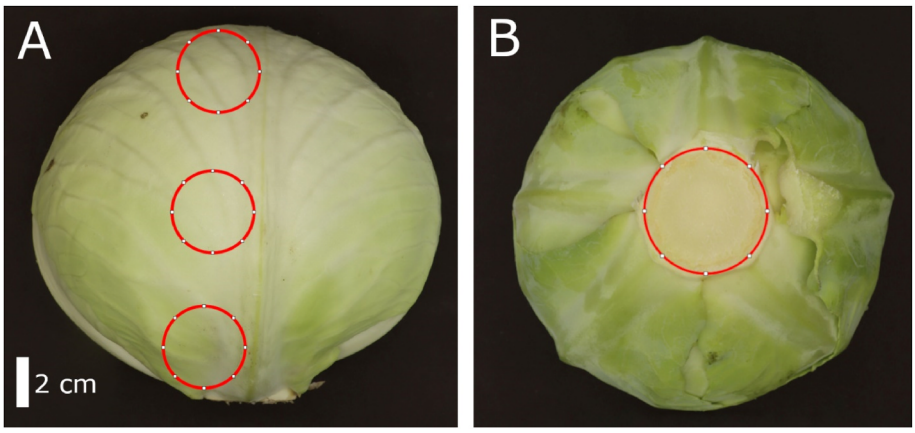


Fig. 1. Head colour sampling approach. Three 3.5 cm diameter circles were sampled across the outer head area (A) whilst the entirety of the stump area was sampled with a fitted ellipse (B).

were incubated on ice for 20 min before centrifugation at 24×*g* for 10 min at 4 °C. The supernatant was evaporated at −52 °C under vacuum in darkness before reconstitution in methanol:water (1:9) with 0.1% formic acid and 1 mM ammonium formate. Samples were analysed via LC/MS-MS using an Agilent 1290 Infinity UHPLC system together with an Agilent 6540 Ultra High-Definition Accurate Mass Q-TOF LC–MS System (Agilent Technologies). Separation was achieved using a 2.1 × 50 mm Hypersil Gold column (Thermofisher, UK) maintained at 30 °C. The mobile phase consisted of (A) water with 0.1% formic acid (v/v) and 1 mM ammonium formate and (B) methanol with 0.1% formic acid (v/v) with 1 mM ammonium formate at flow rate of 0.4 mL min^{-1}. The linear gradient started with 5% B for 0.5 min, then increased to 40% B for 5 min and then to 95% B for a further 1.5 min. These conditions were maintained for 1 min before re-equilibration. PGRs were quantified using external standards (Sigma-Aldrich, UK).

2.4.5. Fermentation products

As a representative fermentation product generating off odours, cabbage head ethanol content was quantified through headspace sampling (HSS) followed by gas chromatography (GC)/time-of-flight mass spectroscopy (TOF/MS) after Landahl et al. (2023). On day 28, samples of fresh tissue (100 g) cut in 2 cm^2 portions were incubated at 55 °C in 0.5L of air for 1 h before HSS using a Tenax SuliCarb thermal desorption tube (Markes International, UK) with a flow rate of 100 ml min^{-1} for 5 min. Samples were collected from six representative heads per treatment. GC was performed using an Agilent 780A GC fitted with a Rxi-624Sil MS 60 mm × 0.25 mm x 1.4 μm column (Restek Ltd., USA) followed by quantification using a Markes BenchTOF_HD MS (Markes International, UK). Ethanol was quantified using an external standard (Sigma-Aldrich, UK).

2.5. Gene expression

Expression of lipoxygenase (*BoLOX1*), subtilisin protease (*BoSBT*) and the PLATZ transcription factor ORE15 (*BoORE15*) were used as indicators of senescent gene activity (Gómez-Lobato et al., 2012; Kim et al., 2018). Pheophytinase (*BoPPH*) was used as an indicator for chlorophyll catabolism (Aiamla-or et al., 2012). Total RNA extraction was performed using the RNeasy Plant Mini Kit (Qiagen, UK), including on-column Dnase treatment. cDNA synthesis was performed using Superscript™ II (Invitrogen, UK) after verification of RNA integrity using agarose gel electrophoresis and 260/280 nm spectrophotometry. RT-qPCR was carried out using a CFX96™ RT-PCR (Bio-Rad, UK) using SsoAdvanced™ Universal SYBR® Green (Bio-Rad, UK) and gene specific primers. For expression analysis, normalisation against β-Actin was performed using the $^{\Delta\Delta}$Ct method. Target sequences and gene specific primers are given in Table S1. Analysis was performed for triplicate biological replicates taken from separate RNA extractions, each with triplicate technical replicates.

2.6. Statistical analysis

Statistical analysis was performed using Genstat for Windows v. 22.1.0.532 (VSNI Ltd., UK). Analysis of variance (ANOVA) suitable for the experimental design (e.g. repeated measures ANOVA for respiration) for the *treatment, temperature, day* factors and their interactions was calculated. ANOVA assumptions were tested and found to be valid for this dataset. Least significant differences (LSD) were calculated and provided on the figures preferentially for the triple *treatment*temperature*day* interaction, or for other factors/interactions that were found to be significant ($p = 0.05$) where appropriate.

3. Results

3.1. Respiration

Respiration rate, as CO_2 production, stabilised within five days of storage until the end of the experiment (Fig. 2). Temperature had a suppressive effect on respiration, with Control/5 °C heads showing an

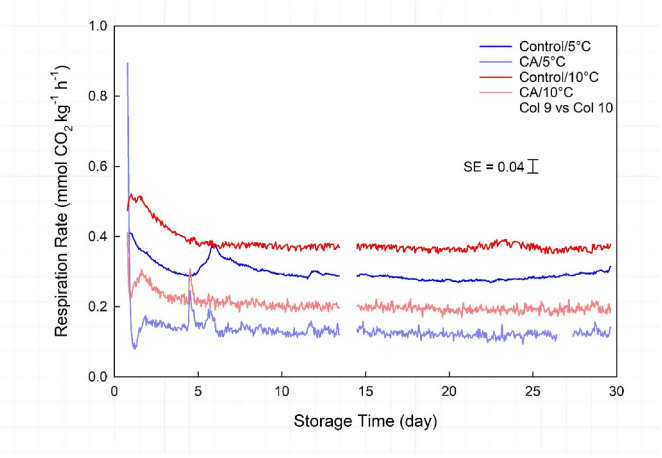


Fig. 2. Respiration rate reported as mmol CO_2 kg^{-1} h^{-1} of cabbage during 28 days of storage at 5° and 10 °C under two gaseous environments: control (air) or controlled atmosphere (CA; 1.5 kPa CO_2 and 6 kPa O_2). Average values are plotted ($n = 3$), and the vertical bars indicated the Standard Error (SE) as calculated through repeated measures ANOVA.

average reduction of 21.6% compared with Control/10 °C. CA treatment had a more profound effect on respiration, with a reduction of 46.3% and 66.2% in average respiration rates under CA/10 °C and CA/5 °C respectively, compared to Control/10 °C heads.

3.2. Head quality

Control/10 °C heads underwent substantial colour changes that were absent in heads stored at 5 °C or CA/10 °C. C* values increased during storage in all treatments except for Control/10 °C, which was reduced by 4% throughout storage (Fig. 3A), with *treatment*temperature*day* having a significant effect ($p = 0.029$). °h declined during storage, with the greatest reduction seen under Control/10 °C of 3.8% by day 28 (Fig. 3B). h° also showed a significant response to *treatment*temperature*day* ($p = 0.047$). L* values initially increased during storage before stabilising for the remainder of the experiment, with the greatest increase seen in Control/10 °C heads increased by 44% by day 21 although only the *treatment*day* interaction was significant ($p < 0.001$; Fig. 3C).

Heads stored under Control/10 °C showed extensive adventitious root formation, with roots exceeding 1 mm recorded on day 21 (average length 11.8 mm, roots present on 100% of heads), increasing to 17.5 mm average length on day 28 (Fig. 4). One third of the heads stored under Control/5 °C showed root formation by day 28, with an average root length of 3.1 mm. Stumps whitened during initial storage, with an increase in C* until day 14 in all treatments (Fig. 5). This was maintained at 5 °C, where C* were increased by 25–26% by day 28. However, heads stored at 10 °C underwent stump darkening in later storage, with a reduction of 27.6% and 4.5% in C* under Control/10 °C and CA/10 °C respectively by day 28, with stump C* showing a significant response to the *treatment*temperature*day* interaction ($p = 0.03$).

Head weight loss was only significantly impacted by temperature ($p = 0.001$), with average weight loss at 5 °C (3.24%) reduced relative to 10 °C (4.77%; Fig. 6).

3.3. Pigments

Total chlorophyll concentrations fluctuated during storage (Table 1), showing only significant effects of *treatment*temperature* and *temperature*day* ($p = 0.007/0.05$). Despite this fluctuation, Control/10 °C heads showed a sustained decrease in total chlorophyll, reaching a 71.3% reduction by day 21, which was not observed in other treatments. The Chl*a*:*b* ratio increased by 66.1% in Control/10 °C treated heads by day 28, with the *treatment* factor solely responsible for these changes ($p = 0.028$). β-carotene concentrations varied during storage, although levels were consistently lower in Control/10 °C compared with the other treatments, with reductions of 32% in Control/10 °C by day 28. All double interactions were significant for β-carotene. Lutein concentrations increased under CA treatment by 119.5% and 126.2% at day 28 under CA/5 °C and CA/10 °C respectively, whilst a decline of 30.3% under CA/10 °C was observed. Both *treatment*temperature* and *treatment*day* interactions were significant in this case ($p = 0.008/0.015$ respectively).

3.4. Organic acids

Malate concentrations increased during storage, particularly Control/10 °C treatments where levels increased by 45.8% by day 21 whilst other treatments saw only marginal changes (Fig. 7A). However, differences were less pronounced by day 28, and only *treatment* and *temperature* factors had a significant effect on malate concentrations ($p = 0.03$). Ascorbate levels fluctuated during storage (Fig. 7B), and while all treatments demonstrated a reduction by day 28 this was greatest in Control/10 °C treated heads (23.6%) whilst CA/10 °C were comparable with both 5 °C treatments (17.0% on average); only *day* showed a significant effect ($p = 0.01$). Citrate showed a significant effect of *treatment*temperature*day* ($p = 0.01$), which showed transient increases in

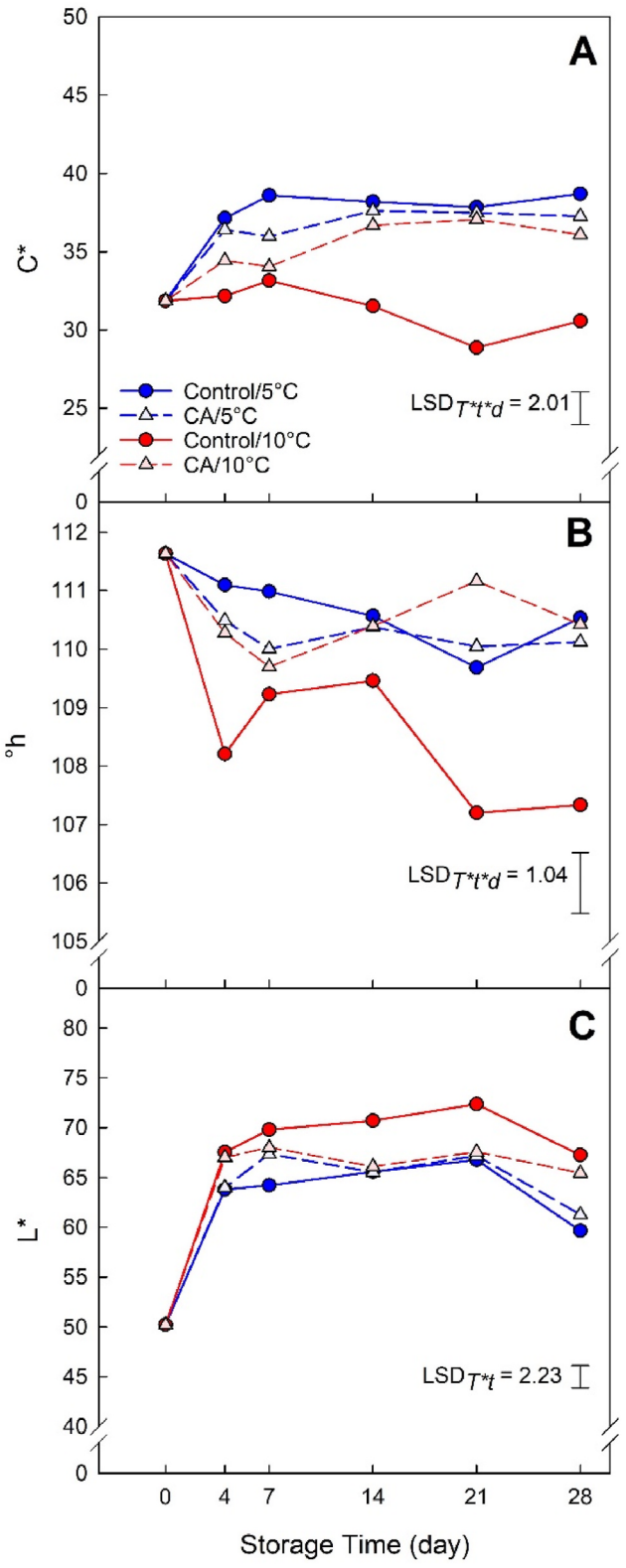


Fig. 3. Cabbage head colour changes reported as Chroma (C*, **A**), Hue angle (°h, **B**) and lightness (*L) during 28 days of storage at 5 and 10 °C under two gaseous environments: Control (air) or controlled atmosphere (CA; 1.5 kPa CO_2 and 6 kPa O_2). Average values are plotted from three replicates ($n = 3$). Vertical bars represent the least significant difference (LSD) for the treatment (*T*), temperature (*t*), day (*d*) factors and their interactions where significant ($p < 0.05$).

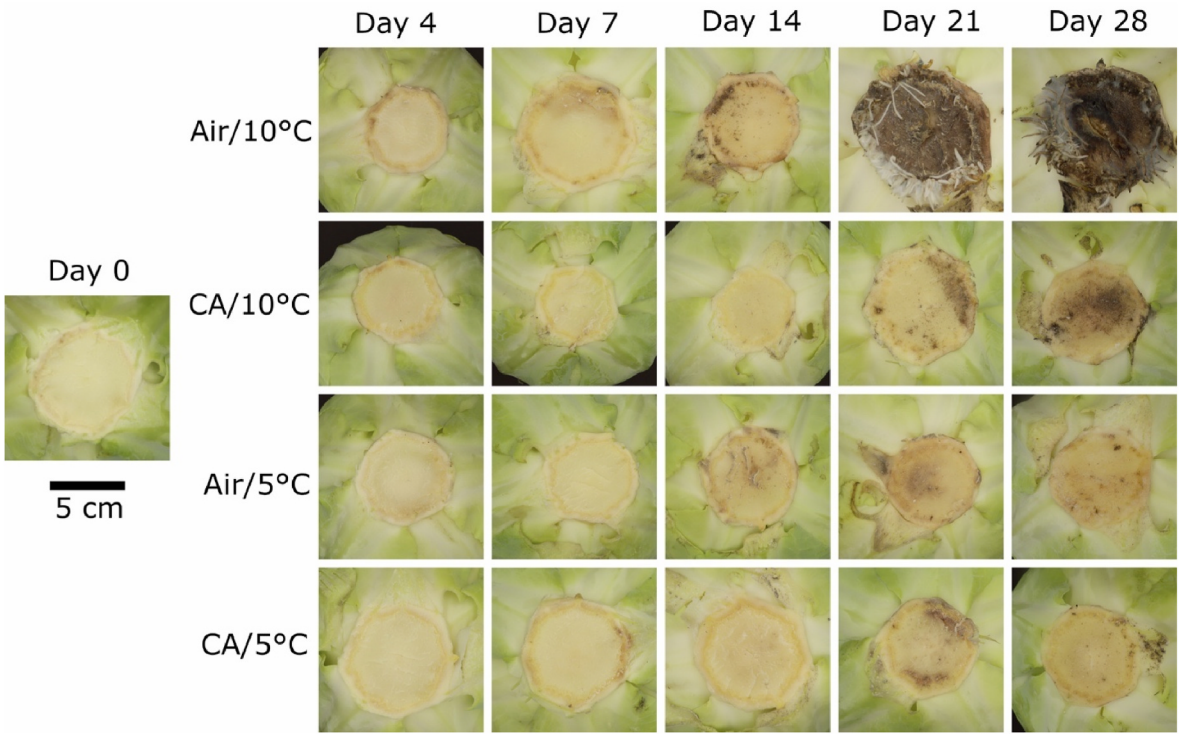


Fig. 4. Images of representative stumps of cabbage stored at 5/10 °C during 28 days under two gaseous environments: Control (air) or controlled atmosphere (CA; 1.5 kPa CO_2 and/6 kPa O_2).

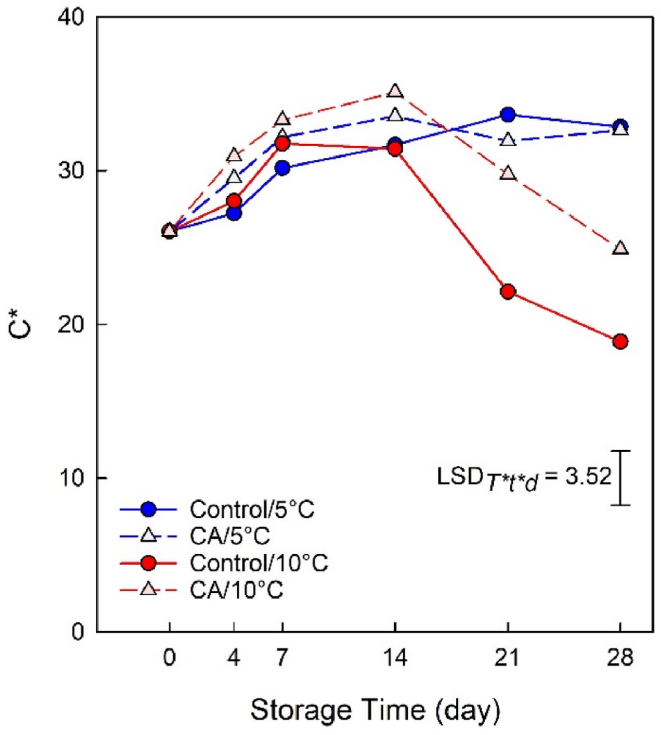


Fig. 5. Cabbage stump colouring in response to under 28 days of storage at 5/10 °C under two gaseous environments: Control (air) or controlled atmosphere (CA; 1.5 kPa CO_2 and/6 kPa O_2) as assessed through Chroma (C*) value. Average values plotted from three replicates ($n = 3$). Vertical bars represent the least significant difference (LSD) for the triple interaction of the treatment (T), temperature (t), day (d) factor ($p < 0.05$).

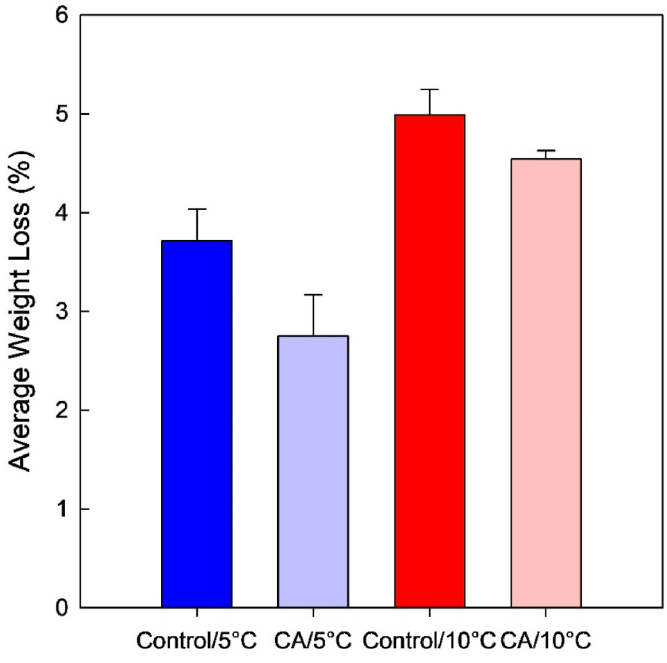


Fig. 6. Average total weight loss in percentage (%) for cabbage heads after 28 days of storage at 5/10 °C under two gaseous environments: Control (air) or controlled atmosphere (CA; 1.5 kPa CO_2 and 6 kPa O_2). Average values plotted from three replicates (n = 3). Vertical bars: standard error of the mean.

Table 1
Pigment concentrations of cabbage stored for 28 days of storage at 5/10 °C under gaseous treatments: control (air) or controlled atmosphere (CA; 1.5 kPa CO_2 and/6 kPa O_2) as assessed through total chlorophyll (Total Chl), chlorophyll *a:b* ratio (Chl *a:b*) and the carotenoids β-carotene and lutein. Average values are given ($n = 3$) together with standard error of the mean. Least significant difference (LSD) values are given in italics ($p < 0.05$) except where factors were not significant (−). Values for Chlorophyll *a and b* are given separately in **Supplementary Information 2**.

		Storage Time (Day)					
		0	4	7	14	21	28
Total Chl (g kg^{-1})	Control/5 °C	9.7 ± 0.86	20.3 ± 6.5	12.1 ± 4	9.0 ± 1.6	11.4 ± 4	19 ± 2.5
	CA/5 °C		12.1 ± 4.5	7.8 ± 0.0	6.3 ± 0.9	12.9 ± 1.5	11.7 ± 1.7
	Control/10 °C		15.4 ± 7.6	7.7 ± 0.5	5.8 ± 1.4	2.8 ± 0.4	3.5 ± 0.6
	CA/10 °C		10.2 ± 1.2	8.6 ± 2.9	17.3 ± 2.3	11.5 ± 3.1	7.0 ± 0.7
Chl *a:b*	Control/5 °C	1.9 ± 0.02	1.4 ± 0.3	2.7 ± 1.5	3.1 ± 1.3	2.4 ± 0.8	1.1 ± 0.0
	CA/5 °C		2.6 ± 0.7	0.9 ± 0.3	3.0 ± 0.8	1.6 ± 0.1	1.7 ± 0.3
	Control/10 °C		2.3 ± 0.5	1.1 ± 0.4	2.8 ± 0.7	4.5 ± 0.4	3.1 ± 1.1
	CA/10 °C		1.4 ± 0.1	1.4 ± 0.7	1.6 ± 0.2	1.5 ± 0.2	2.0 ± 0.1
β-carotene (g kg^{-1})	Control/5 °C	43.3 ± 4.8	66.7 ± 11.9	54.8 ± 9.6	39.8 ± 4.1	49.8 ± 8.5	53.2 ± 6.6
	CA/5 °C		59.4 ± 4.7	41.8 ± 1.2	38.6 ± 3.5	52.4 ± 3.5	43.4 ± 1.8
	Control/10 °C		70.3 ± 18.3	35.1 ± 3.8	33.7 ± 3.7	22.9 ± 3.2	29.4 ± 1.9
	CA/10 °C		47.0 ± 5.8	47.6 ± 8.8	79.5 ± 19.1	46.9 ± 6.9	35.2 ± 3.1
Lutein (g kg^{-1})	Control/5 °C	145.1 ± 31.76	308.1 ± 70.2	256.7 ± 44.6	186.4 ± 19.3	173.2 ± 28.6	204.5 ± 16.8
	CA/5 °C		323.4 ± 6.5	357.4 ± 37.5	314.9 ± 30.9	337.0 ± 8.8	318.4 ± 10.7
	Control/10 °C		306 ± 84.3	155.9 ± 12.6	154.4 ± 20.5	84.2 ± 12.6	101.1 ± 5.6
	CA/10 °C		317.4 ± 35.9	405.1 ± 85.6	510.9 ± 133.4	363.2 ± 39.7	328.2 ± 19.1

LSD	Total Chl	Chl *a: b*	β-Carotene	Lutein
Day	-	-	*18.1*	-
Temperature	*6.7*	-	-	-
Treatment	-	*1.4*	-	*101.4*
*Day*Temperature*	*7.8*	-	*19.8*	-
*Treatment*Day*	-	-	*19.8*	*118.4*
*Treatment*Temperature*	*7.0*	-	*17.7*	*105.9*
*Treatment*Temperature*Day*	-	-	-	-

CA/10 °C (50%, day 7) and Control/5 °C (53.9, day 14; Fig. 7C) although no clear trend was present in citrate accumulation.

3.5. Non-structural carbohydrates

Glucose concentrations initially increased in all treatments before stabilising with no significant changes after day 7 (Fig. 8A). Control/5 °C saw the smallest initial increase (6.8% compared with 12.5–15.4% in the other treatments) although later increases by day 28 resulted in concentrations comparable with other treatments. Glucose presented significant effects from the *treatment*temperature* and *temperature*day* interactions ($p = 0.045/0.08$). Similarly, fructose saw an initial increase until day 7 that was similar between treatments (17.9–20.1%) except for Control/5 °C, which increased only by 11.2% (Fig. 8B). After day 7, levels fluctuated slightly, but remained relatively constant until day 28, with only the *treatment*temperature* interaction being significant ($p = 0.005$). Conversely, sucrose concentrations saw large reductions on day 4, with the greatest reduction of 51.6% in Control/10 °C treated heads (Fig. 8C). After day 14 sucrose concentrations partially recovered under CA treatment, with CA/5 °C and CA/10 °C reaching 65.7% and 79.9% of day 0 levels respectively. Sucrose saw significant effects only from the *treatment*temperature* and *treatment*day* interaction ($p = 0.01/0.004$).

3.6. Plant growth regulators

ABA concentrations rose ca. 3.5-fold in Control heads, increasing by ca. 3-fold for Control/10 °C on day 14 and by 348.3% for Control/5 °C on day 21 (Fig. 9A). Conversely, CA treatment gave immediate reduction in ABA, which remained low for the duration of the experiment with day 28 levels 27% and 23.8% less than that of day 0 under CA/5 °C and CA/10 °C respectively. ABA showed a significant response to the *treatment*temperature*day* interaction ($p = 0.048$). ABA-GE and DPA marginally increased with storage in control heads relative to CA treatment irrespective of temperature, and PA concentrations increased with later storage in Control/5 °C heads, whilst 7-OH-ABA and NeoPA showed no change with storage; however, no significant effect of the *treatment*temperature*time* interaction was observed for ABA metabolites (see Supplementary Table 3).

IPR concentrations saw significant reduction of over 70% with CA treatment irrespective of treatment, compared with 4–8% reduction under Control storage (Fig. 9B). However, subsequent reductions in IPR in control heads, and increases in CA-treated heads gave comparable concentrations by day 28, although the *treatment*temperature*day* interaction was significant ($p = 0.006$). CA treatment also saw greater reductions in IPRP during initial storage, with reductions of 48.9% and 57.9% in CA/5 °C and CA/10 °C respectively, double that seen in Control heads. (Fig. 9C). Whilst concentrations increased during later storage, IPRP concentrations in treated heads only became comparable with control heads on day 28, with *treatment, day* ($p < 0.001$) and *temperature* ($p = 0.0032$) having significant effects. cZR concentrations fluctuated during storage and whilst *day* had a significant effect ($p < 0.001$), there was no overarching trend in cZR concentration (see Supplementary Table 3).

3.7. Fermentation products

Ethanol accumulation occurred in all treatments besides Control/10 °C (Fig. 10), with a significant effect of treatment*temperature on concentrations at day 28. The greatest accumulation was seen in CA/10 °C treatment.

3.8. Gene expression

BoORE15 expression reduced significantly from day 0 to day 4 and remained negligible for the rest of the experiment for heads stored under CA at both temperatures, whilst expression was maintained (albeit at a reduced level) for heads stored under air (Fig. 11A). Control/10 °C heads demonstrated marginally greater increases in *BoORE15* expression than Control/5 °C heads, although *temperature* was insignificant with only *treatment* demonstrating a significant effect ($p < 0.001$).

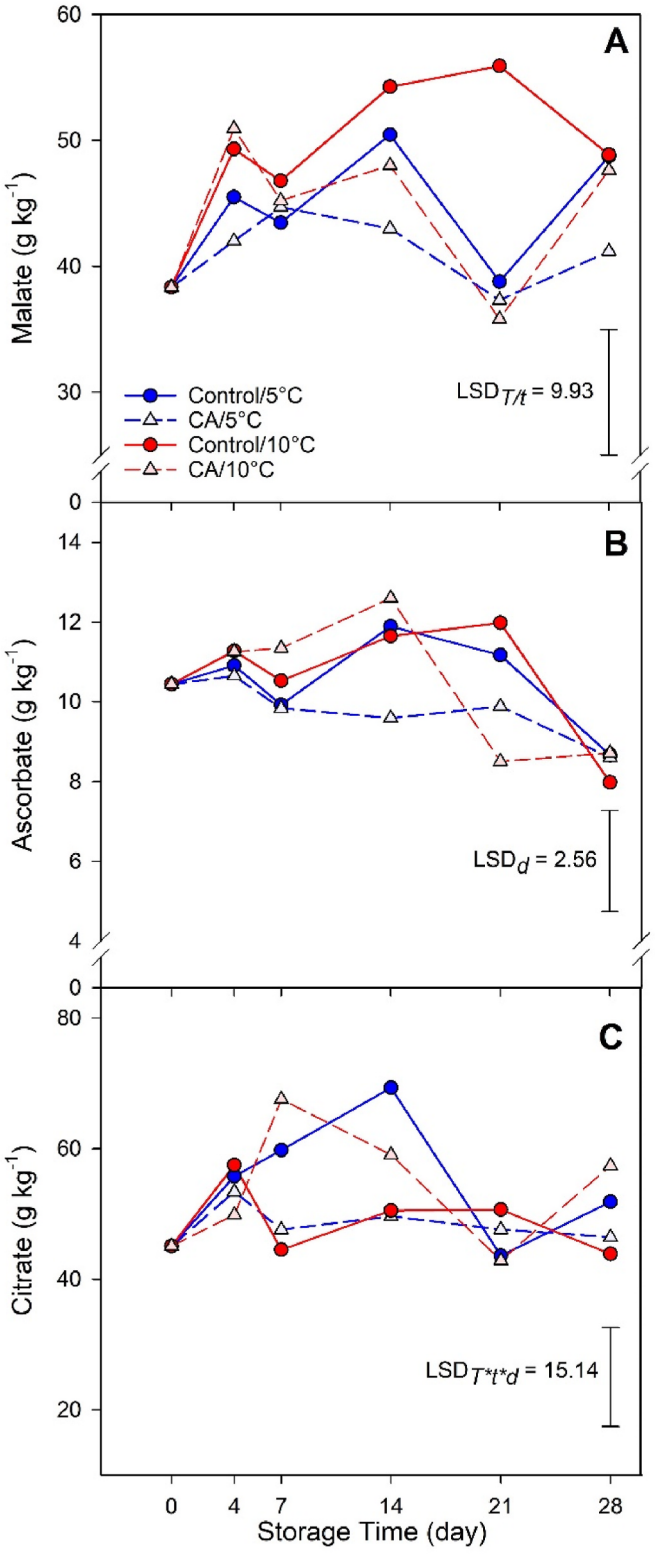


Fig. 7. Organic acid content in g kg^{-1} of cabbage as quantified for malate (A), ascorbate (B) and citrate (C) during 28 days of storage at 5° and 10 °C under gaseous treatments: control (air) or controlled atmosphere (CA; 1.5 kPa CO_2 and 6 kPa O_2). Average values are given ($n = 3$). Vertical bars represent the least significant difference (LSD) for the treatment (T), temperature (t), day (d) factors and their interactions where significant ($p < 0.05$).

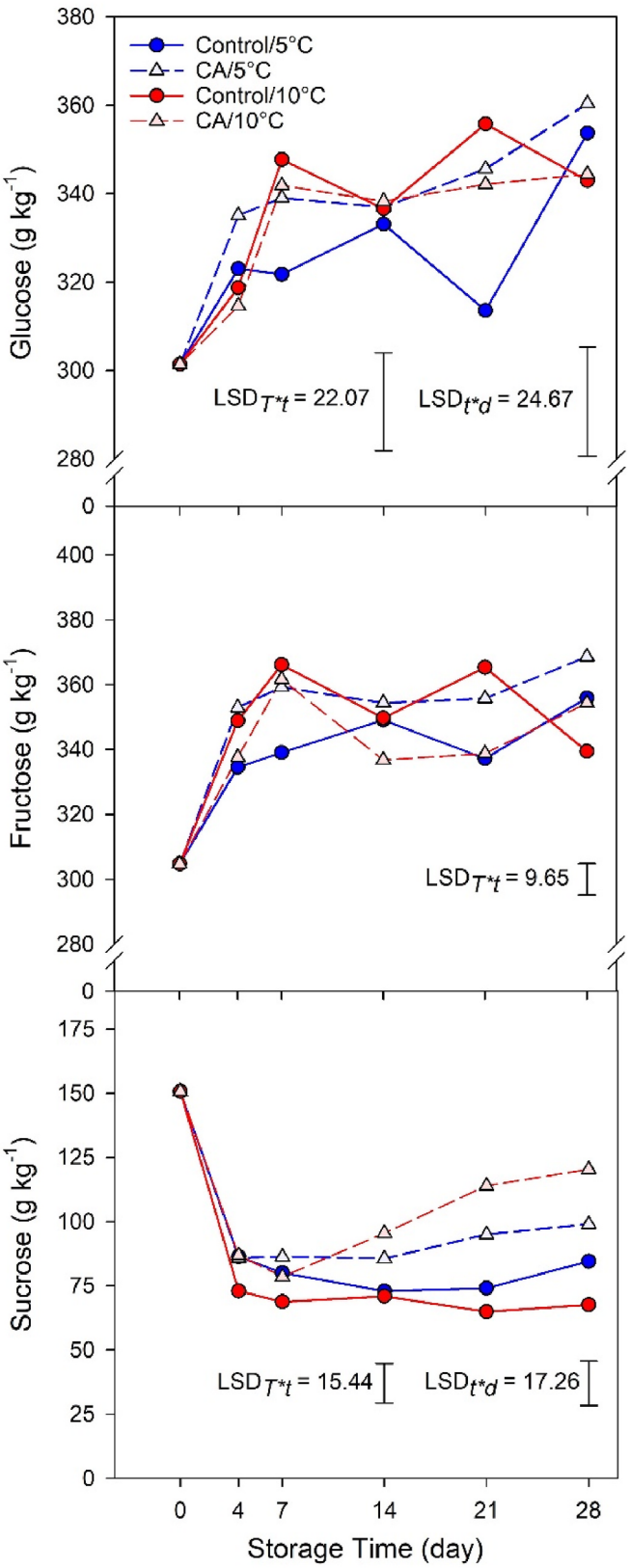


Fig. 8. Sugar content of cabbage as quantified for glucose (A), fructose (B) and sucrose, (C) during 28 days of storage at 5° and 10 °C under gaseous treatments: control (air) or controlled atmosphere (CA; 1.5 kPa CO_2 and 6 kPa O_2). Average values are plotted ($n = 3$). Vertical bars represent the least significant difference (LSD) for the treatment (T), temperature (t), day (d) factors and their interactions where significant ($p < 0.05$).

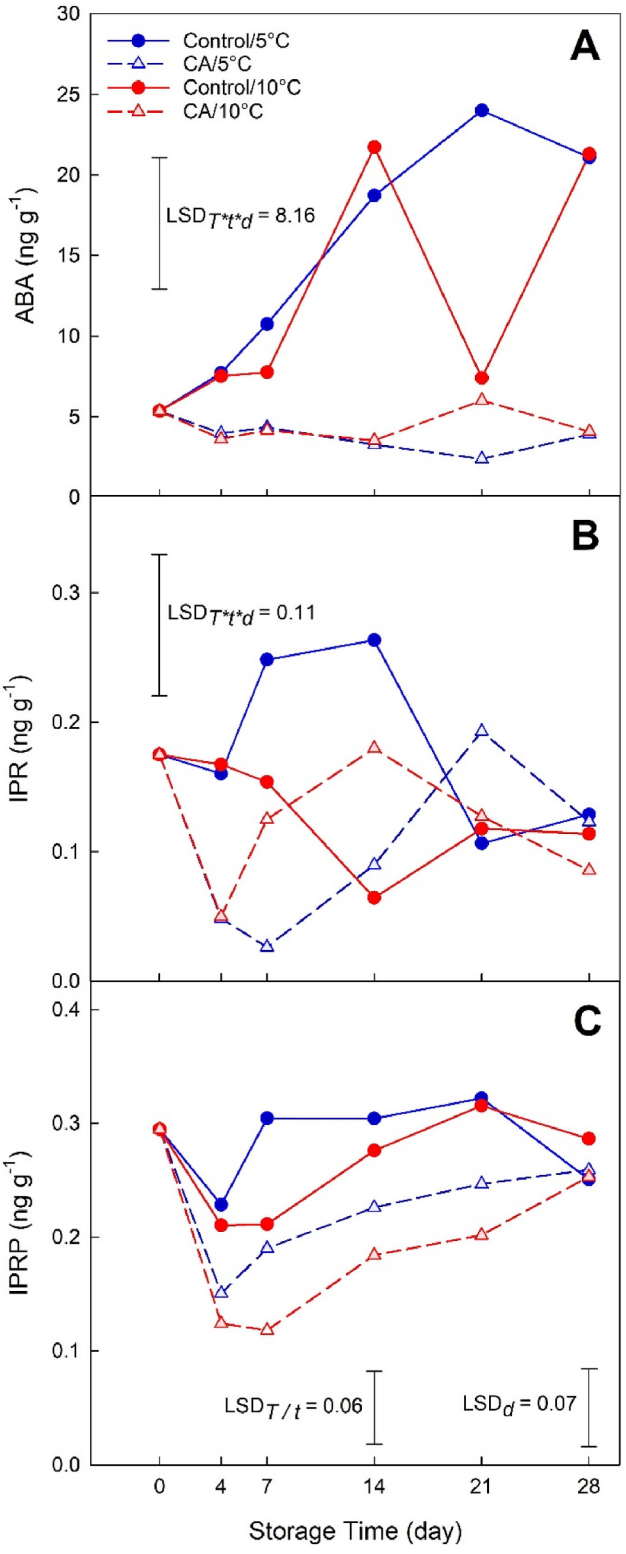


Fig. 9. Concentrations of plant growth regulators in cabbage heads stored for 28 days at 5 and 10 °C under gaseous treatments: control (air) or controlled atmosphere (CA; 1.5 kPa CO_2 and 6 kPa O_2). Concentrations are presented for abscisic acid (ABA, **A**), isopentenyl adenosine riboside (IPR, **B**), IPR phosphate (IPRP, **C**). Average values are plotted ($n = 3$). Vertical bars represent the least significant difference (LSD) for the treatment (T), temperature (t), day (d) factors and their interactions where significant ($p < 0.05$).

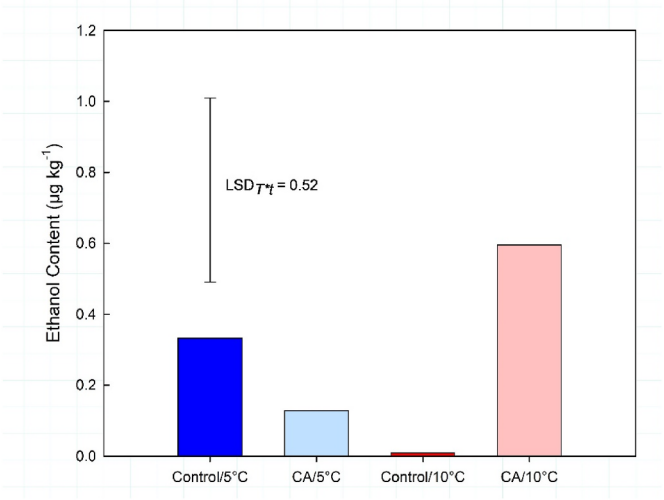


Fig. 10. Ethanol concentration for cabbage heads stored for 28 days at 5 and 10 °C under gaseous treatments: control (air) or controlled atmosphere (CA; 1.5 kPa CO_2 and 6 kPa O_2) quantified through headspace sampling. Average values are plotted ($n = 6$). Vertical bars represent the least significant difference (LSD) for the significant interaction between treatment (T) and temperature (t; $p < 0.05$).

BoLOX1 expression was negligible in all treatments until day 28 when large increases were seen in expression at 10 °C only (Fig. 11B), especially for CA. *BoSBT* expression declined initially but increased from day 7 in heads stored under air, particularly in Control/10 °C heads (Fig. 11C) with a significant effect of the *treatment*temperature*day* interaction ($p = 0.041$). *BoPPH* expression saw significant increases in Control/10 °C stored heads from day 7, although this was delayed until day 21 in CA/10 °C heads (Fig. 11D).

4. Discussion

4.1. Controlled atmosphere slows metabolism at high temperatures

Low temperature storage is a cornerstone of postharvest management because of its ability to suppress respiration and minimise quality loss through retarding catabolic metabolism (Kader and Saltveit, 2002). Increased respiration under elevated temperatures will promote senescence through energy reserve exhaustion causing carbohydrate, protein and chlorophyll loss, modified lipid metabolism and loss of membrane integrity (Able et al., 2005; Ghimire et al., 2023). Respiratory dysfunction will increase oxidative stress by promoting mitochondrial reactive oxygen species production to further promote chlorophyll loss (Yamauchi, 2015). Increased temperatures will also disrupt regulatory coordination, such as increasing production of, and sensitivity to, ethylene furthering senescence (Kramchote et al., 2012; Ghimire et al., 2024). Warmer temperatures can also trigger undesirable quality developments, such as elevated carotenoid accumulation contributing to head yellowing (Luo et al., 2019). Climate change is increasing global temperatures, making it challenging to maintain low temperatures during postharvest storage of fresh produce, increasing the energy demand for refrigeration systems and generating negative environmental impacts associated to greenhouse gas emissions (Gage et al., 2024). CA treatment can support cold storage for respiratory suppression, although evidence examining the interaction between CA and other storage parameters – especially temperature – is still scarce (Saltveit, 2003). Synergy between storage temperature and CA treatment during postharvest has been reported previously (Schouten et al., 2009), but the understanding of the underlying physiology is equally insufficient.

The results of the present study demonstrate that CA treatment can reduce cabbage respiration rates to a greater extent than chilling to 5 °C

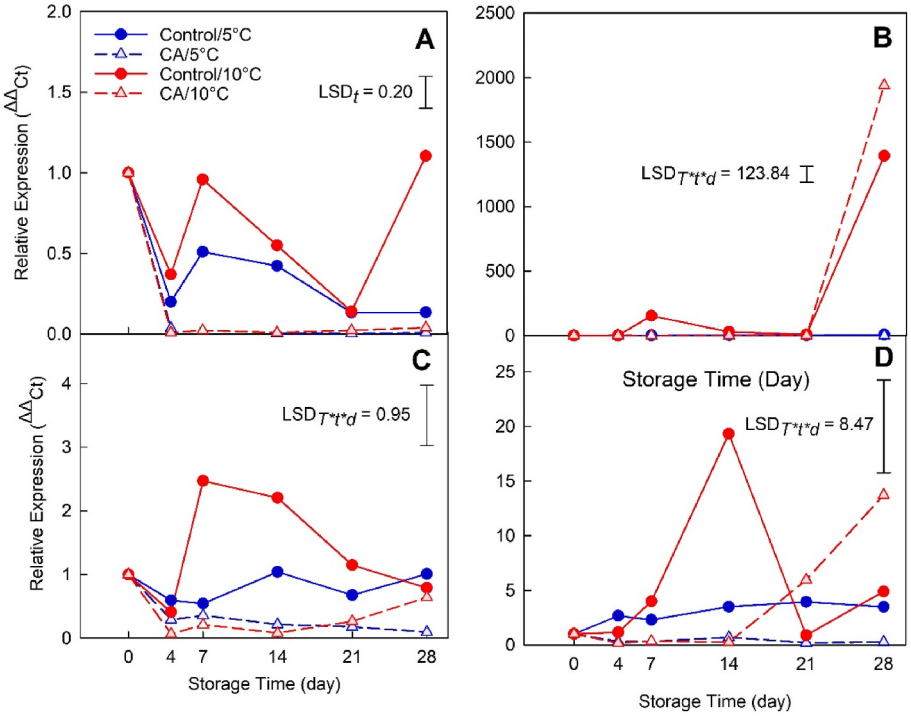


Fig. 11. Changes in relative gene expression in cabbage heads stored for 28 days at 5 and 10 °C under gaseous treatments: control (air) or controlled atmosphere (CA; 1.5 kPa CO_2 and 6 kPa O_2). Changes in expression for *BoORE15* (**A**), *BoLOX1* (**B**), *BoSBT* (**C**) and *BoPPH* (**D**). Average values are plotted (n = 3). Vertical bars represent the least significant difference (LSD) for the treatment (T), temperature (t), day (d) factors and their interactions where significant ($p < 0.05$).

alone. The storage of fresh produce under hypoxic CA conditions will promote rapid changes in metabolic activity to minimise energy consumption and promote efficiency (Geigenberger, 2003). Whilst evidence of hypoxic thresholds in leaf/shoot partitions is scarce, 6 kPa O_2 has been reported to trigger foliar hypoxic stress responses (He et al., 2007) and therefore we consider that the CA treatment used here is liable to trigger hypoxic stress in cabbage, particularly given the apparent onset of fermentation under CA conditions. Alternative respiratory pathways can be promoted under restricted O_2 availability, including increased glycolytic activity (Liu et al., 2005; Li et al., 2016) alongside modification of expression profile of genes associated with carbohydrate metabolism, particularly pentose and glucuronate conversion pathway (Ghimire et al. (2024). Results in the present study demonstrated that the metabolic shift under CA conditions was maintained at 10 °C. The accumulation of malate under Control treatments is indicative of reduced carbon flow through the Szent-Györgyi-Krebs cycle (Launay et al., 2019) likely resulting from respiratory substrate exhaustion, although this was suppressed by CA treatment. Conversely, CA treated heads showed an increase in sucrose concentration, which may imply improved carbohydrate efficiency under CA conditions, or respiratory changes under hypoxia resulting in conversion from invertase to sucrose synthase in glycolysis to improve ATP efficiency (Li et al., 2016). This contrasts with other brassicas, where CA reduced sucrose in broccoli whilst having no effect on cauliflower (McKenzie et al., 2004; Hodges et al., 2006). This difference may be due to reduced respiration rates in cabbage (which are liable to have become dormant at maturity) compared with developing floral tissue or linked to increased sucrose accumulation following remobilisation of internal starch reserves (Nilsson, 1993). The accumulation of ethanol under CA/10 °C indicates the onset of hypoxic stress, and that metabolic modifications are insufficient to fully mitigate hypoxia stress, resulting in the onset of fermentative metabolism following compromised mitochondrial respiration. This corresponds with Menniti et al. (1997), who reported ethanol accumulation during storage under 3 kPa O_2/10 kPa CO_2. However, the presence of ethanol under Control/5 °C treatment may indicate background levels of fermentation during storage (potentially due to restricted airflow into interior layers resulting in localised hypoxia), and so the observed change under CA/10 °C may not be attributable to treatment effects alone. Ethanol accumulation may reduce quality, although Hansen et al. (2001) reported that ethanol accumulated during CA storage dissipated rapidly during post-storage aeration, which would occur once heads entered the supply chain. Reduced respiration under CA also enhanced quality retention as the significant declines in head quality seen under Control/10 °C were prevented by CA treatment. The increased reduction in chlorophyll concentrations in Control/10 °C heads was likely to have contributed to the reduction in C* and °h as other treatments demonstrated maintenance of both chlorophyll and head colour. Similar trends were seen in the carotenoids β-carotene and lutein, which showed increased maintenance under CA relative to the Control/10 °C treatment. The variability observed in head chlorophyll content, along with the limited correlation between this chlorophyll content and °h in our results, is likely linked to differences in the extent of outer leaf senescence or disease during overwintering. Since the interiors of the heads are self-etiolated, chlorophyll is present only in the outermost leaf layers. Furthermore, the accumulation of oxidative stress-related carotenoids (e.g. β-cryptoxanthin and zeaxanthin) have been reported as contributory to yellowing in broccoli (Luo et al., 2019), and may explain why the extent of colour change in Control/10 °C outpaces the observed changes in chlorophyll content.

CA treatment also minimised quality loss through changes in stump condition, which saw significant declines under Control/10 °C treatment due to adventitious rooting and blackening. Blackening was likely attributable to disease activity (e.g. *Alternaria* ssp. or *Stemphylium* ssp.), and whilst our results indicate a suppressive effect on CA on pathogen growth, this may vary between causative agent (Barkai-Golan, 1990) and requires further exploration as the relationship between CA treatment and disease suppression is poorly understood. The suppressive effect of CA on adventitious rooting may be due to a combination of effects through metabolic suppression, or alleviation of stress perception and hormonal imbalance due to harvest wounding or postharvest abiotic

stress (Torres-Contreras et al., 2018).

4.2. Hypoxia responses delays senescence

Beneficial effects of CA are also attributable to changes in hormonal regulation, especially reduction in ABA accumulation under CA. ABA accumulation can promote senescence such as enhancing chlorophyll catabolism (Gao et al., 2016) and therefore, reduced ABA accumulation under CA has been linked with enhanced quality retention in this study and Chinese cabbage and asparagus as reported by Wang and Ji (1988) and Anastasiadi et al. (2020).

Increased ABA under air storage may result from accelerated metabolism. Wounding responses after harvest can promote upregulation of ABA- and ethylene-derived stress responses (Kato et al., 2002), which may be prevented by metabolic reduction under CA treatment. Anastasiadi et al. (2020) attributed ABA accumulation to postharvest dehydration stress, which was offset by CA treatment, and the onset of rooting may increase stress-related ABA accumulation due to increased vulnerability to e.g. water and salinity stress of developing root primordia (Agulló-Antón et al., 2014; Sarkar et al., 2023). Carotenoid changes in the present study may also correlate with changes in ABA accumulation, as catabolism of β-carotene to zeaxanthin is promoted by oxidative stress due to its role in the protective xanthophyll cycle and as a direct precursor of ABA following carotenoid catabolism in late senescence (Duan et al., 2012). Suppression of these processes under CA conditions can contribute to a reduction of ABA in CA storage. However, the lack of a temperature response to ABA accumulation may imply that the reduction of ABA under CA conditions is due to modified stress responses, particularly in response to hypoxia. ABA acts as a regulator of plant responses under low oxygen conditions, with rapid onset of ABA reductions occurring under conditions of hypoxia (Wang et al., 2021a). Downregulation biosynthesis and enhanced catabolism of ABA occurs under hypoxic conditions as part of wider regulatory changes underpinning adaptation to low oxygen environments (González-Guzmán et al., 2021), with downregulation of hormonal signal transduction genes reported under CA in broccoli (Ghimire et al., 2024). The onset of hypoxic stress under CA treatment also corresponds with the increased sucrose accumulation under CA conditions. Hypoxic respiratory adaptations promote sucrolysis via sucrose synthase as a precursor to glycolysis as a low energy alternative to invertase (Mustroph et al., 2009), and so the observed changes in sucrose concentrations under CA may also be indicative of hypoxic stress. Therefore, we propose that the reduction in ABA is due to perception of hypoxic stress under CA conditions, which feeds forward into improved maintenance of quality through reduction in ABA-mediated senescence.

Interestingly, CA treatment appears to negatively affect cytokinin accumulation given the observed reductions in IPR and IPRP. Cytokinin accumulation has been associated with enhanced postharvest longevity through reduced chlorophyll catabolism and ethylene sensitivity in brassica (Gapper et al., 2005). Crosstalk between carbohydrate and cytokinin signalling (Wang et al., 2021b) may contribute to changes in cytokinin accumulation under the observed changes in sucrose content with CA treatment. Whilst cytokinins play a regulatory role in mediating stress responses, their role in hypoxic stress is not well understood. However, downregulation of cytokinin biosynthesis and upregulation of catabolic cytokinin oxidases have been reported under hypoxia (Cukrov et al., 2016; He et al., 2023). Therefore, metabolic reduction under CA treatment may also be achieved through reduction in cytokinin-mediated activity. Raines et al. (2016) reported cytokinin-response factors that act as positive regulators of senescence, and therefore the reduction in cytokinin activity may have beneficial effects for leaf longevity. However, until greater clarity is reached regarding the interaction between cytokinin, CA conditions and result physiological modification it is not possible to appraise the origins or effects of the observed changes in cytokinin concentration.

4.3. Controlled atmosphere modifies senescent gene regulation

The mechanisms behind CA capacity to delay senescence can be explained on a regulatory level directly. Warmer storage temperatures increased *BoORE15* expression, which were maintained throughout cabbage senescence (Ahlawat and Liu, 2021). While this contracts with our results, which showed a reduction in *BoORE15* with later storage, it aligns with other studies that suggest *ORE15* plays a greater role in leaf development and early senescence (Kim et al., 2018). Our results also showed that CA had a suppressive effect on *BoORE15* expression, and that senescence was reduced at a regulatory level. Downstream effects of suppressed senescent activity were also evident in *BoSBT* expression, which showed increased expression under air and under warmer temperatures, whilst being significantly reduced by CA treatment. Leaf senescence can be seen to promote elimination of inefficient older leaves to conserve nutrients internally, with increased postharvest senescence in pak choi (*B. rapa* subsp. *chinensis*) under warmer temperatures demonstrating increased activity of nitrogen remobilisation activity (Dhandapani et al., 2023). A reduction in senescent metabolism is correlated by the expression profiles of both *BoPPH* and *BoSBT,* which show increased expression with Control/10 °C treatment but show the greatest reduction under CA treatment. Pheophytinase (*BoPPH*) expression also correspond with reduced senescent behaviour under CA treatment given its role in chlorophyll catabolism (Aiamla-or et al., 2012). CA treatment was able to delay the significant increase in *BoPPH* expression seen at 10 °C, indicating that the reduction in chlorophyll in Control/10 °C heads resulted from regulatory changes rather than suppression of catabolic metabolism. In addition to showing the strongest correlation with visual colour change in broccoli (Büchert et al., 2011), changes in PPH expression coincide with the expression profiles of other components of the senescent chlorophyll degradation pathway in Brassica (Zhang et al., 2011; Song et al., 2020). Therefore, these observed changes are considered indicative of broader regulatory modifications of chlorophyll degradation under CA treatment. *BoLOX1* showed a similar response to temperature, with its expression completely suppressed by storage at 5 °C whilst a significant increase was seen in expression on day 28 for 10 °C treatments. However, CA treatment significantly increased *BoLOX1* expression compared with the air control at 10 °C. Gómez-Lobato et al. (2012) reported that whilst *BoLOX1* expression was reduced in broccoli under CA, total lipoxygenase activity was increased. Given that CA storage is liable to have a modifying effect on changes to microsomal membrane integrity (Deschene et al., 1991), it is possible that *BoLOX1* activity is impacted by physiological changes distinct from wider control of senescent metabolism. Given the relatively minor biochemical seen in response to CA treatment and the apparent lack of respiratory dysfunction the large-scale changes in gene expression are not entirely explained. Further evidence as to the regulatory background of these bioindicators is necessary in clarifying the relationship between CA treatment and the genetic control of senescence.

5. Conclusions

We observed how hypoxic conditions can trigger wider changes in hormonal control and underlying gene regulatory networks to further reduce quality loss, with the suppression of ABA accumulation likely to contribute to reduced postharvest quality loss. Our results demonstrated that it is possible to increase storage temperature of cabbage if postharvest management is supported with CA as it was able to delay senescence through reduction of catabolic metabolism even at 10 °C. Our study focused on late-harvest cabbage, and results may not be comparable with other varieties which are harvested earlier with greater proportions of mature green leaf (e.g. Savoy). Our results provide key insights for an alternative practice of cabbage postharvest handling in the supply chain, whilst providing further evidence as to the effect of CA on postharvest physiology. The ability to store cabbage at warmer temperatures under CA treatment may also contribute to progress

towards reducing carbon emissions, therefore enhancing the sustainability of the supply chain whilst reducing losses through improved postharvest storage techniques.

Data availability statement

Data supporting this study are openly available from Cranfield University online research depository at DOI: https://doi.org/10.57996/cran.ceres-2617.

CRediT authorship contribution statement

Ewan Gage: conceptualization, investigation, data curation, formal analysis, writing - original draft, writing – review & editing; **Ritika Jain:** investigation, writing – original draft; **Leon A. Terry:** funding acquisition, writing – review & editing; **Natalia Falagán:** conceptualization, funding acquisition, supervision, writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data is uploaded to a public repository and the DOI will be included in the paper as soon as it is ready prior to publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2024.109094.

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