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Optimisation of the controlled atmosphere storage and shelf life of *Lilium longiflorum*

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ORIGINAL ARTICLE

Abstract

To prolong the storage period of fresh *Lilium longiflorum*, maintain its quality during storage, and optimise the controlled atmosphere (CA) storage parameters. In a single-factor experiment, the temperature, humidity, and O_2 and CO_2 concentrations were considered as primary factors affecting the CA storage of *L. longiflorum*. Then, a comprehensive score was optimised using a back propagation neural network combined with empirical data. Finally, experimental verification was undertaken. The optimal concentrations of O_2 and CO_2 were 4.5% and 3.8%, respectively. A storage temperature of 4.2°C and a relative humidity of 90% were ideal. Under these conditions, the comprehensive evaluation score for *L. longiflorum* was 0.8424 (P > 0.05), consistent with the predicted value of 0.8372. Compared to ordinary cold storage, the storage period of *L. longiflorum* under these CA storage conditions was effectively prolonged. This provided an experimental basis for the CA storage of *L. longiflorum*.

Keywords: Lilium longiflorum; comprehensive evaluation; back propagation neural network; genetic algorithm; controlled atmosphere storage

Introduction

Lilium longiflorum is a respiratory climacteric plant belonging to genus *Lilium* of family *Liliaceae*. It is a popular commercial food product favoured for its nourishing yin, lung-moistening effects, and anti-inflammatory and anti-tumour properties (Li *et al.*, 2020; Zhou *et al.*, 2021). According to Zhao *et al.* (2018), *L. longiflorum* is sold as fresh fruit in the Chinese markets, accounting for approximately 90% of the total annual output. However, *L. longiflorum* has high water content and a vigorous metabolism, making it prone to browning, atrophy, and rotting after harvest, seriously affecting its market value. Therefore, it is of great scientific and economic significance to optimise a long-term storage method for *L. longiflorum* to provide high-quality fresh products to consumers year-around.

Currently, the Chinese market commonly employs low-temperature and modified/controlled atmosphere (CA) storage technologies. Low-temperature preservation is favoured for fast-selling fruits and vegetables because of its low cost and easy maintenance. Although low-temperature preservation is China's most widely used technology, it is less effective for fruits and vegetables that require long-term preservation. Atmosphere manipulation techniques are today's most mature and commonly used global storage technologies. The principle is to reduce the metabolic rate of fruits and vegetables by manipulating storage environment, which typically involves controlling of temperature, humidity, and air composition to prolong the storage period (Thewes *et al.*, 2017). The two main types are modified atmosphere (MA) storage and CA storage (Ye *et al.*, 2021). These strategies involve manipulating many factors to improve preservation, with the cost of infrastructure for CA being much higher than low-temperature storage. Although the cost of CA vesus cold storage (CS) is high, the storage effects are also excellent. Ho *et al.* (2020) used CA to store dragon fruit. The study's results showed that CA storage lessened loss of flavour in substances, kept good fruit acidity and sensory quality levels, and prolonged the storage period. Bender *et al.* (2021) attained similar results, showing that mango stored in CA did not produce chilling injuries at lower temperatures, effectively prolonging its storage period. Xiao *et al.* (2020) compared *Codonopsis pilosula* using natural storage, conventional sealing, and CA storage methods for 12 months, observing that the effective components of *Codonopsis pilosula* stored in CA had the best preservation effects. However, in China, research on the preservation of *L. longiflorum* has focused on low-temperature refrigeration and MA storage (Li *et al.*, 2020; Zhang *et al.*, 2020), while research on CA storage has not been reported yet.

Response Surface Methodology (RSM) is an optimisation method that combines mathematical statistical modelling and experimental design (Chouaibi *et al.*, 2020; Hundie *et al.*, 2020). RSM can be used to determine the optimal experimental values of independent and dependent variables. The main advantage of RSM is that fewer test points are required to obtain acceptable results, thereby saving substantial time and material. However, when RSM uses fixed higher-order equations to analyse experimental data, it results in some information loss and reduced accuracy. Back propagation neural networks (BP-NN) are a learning process of artificial intelligence (AI), and are widely used for data analysis in food science and agriculture. BP-NN can achieve a good simulation effect in the face of complex systems, such as in predictive models of post-harvest peach pre-cooling effectiveness (Chen *et al.*, 2021), quality changes in wheat during storage (Jiang *et al.*, 2021), and extraction of polysaccharide substances (Li *et al.*, 2021). However, BP-NN approaches cannot design experiments and easily fall into local extrema during data processing (Yu *et al.*, 2021). In order to overcome these defects, BP-NN approaches are usually based on other experimental designs to obtain data for analysis. For example, they are often used in conjunction with genetic algorithms (GA) to identify the global minimum (or maximum) values (Ghaedi and Vafaei, 2017) and avoid artificial neural networks (ANN) falling into a local extremum. The current study found that experimental results analysed by a genetic algorithm-neural network (GA-NN) technique were superior to results of RSM for root mean square error (RMSE), mean absolute percentage error (MAPE), and R^2, indicating that GA-NN technique is more effective than RSM for data analysis (Yin *et al.*, 2021). Fuzzy mathematics is a mathematical method that studies and handles fuzzy phenomena (Liao *et al.*, 2017; Xue and He, 2021). The current study had many indicators of changes in *L. longiflorum*, making it difficult to accurately describe the current state of *L. longiflorum* using a single index. Fuzzy mathematics was used to process multiple evaluation indices concerning *L. longiflorum*, and a comprehensive evaluation index was calculated to summarise the physiological state of *L. longiflorum*.

This study aimed to apply AI and CA technology to investigate the storage environment of *L. longiflorum.* Based on a single factor, this study adopted an RSM experimental design, optimising the comprehensive evaluation constructed by fuzzy mathematics through GA-NN to obtain optimal values for various parameters involved in the CA storage of *L. longiflorum.* The determined optimum storage environment was then compared with commonly used 4°C cold storage. Finally, O_2 and CO_2 concentrations, temperature, relative humidity (RH) of the package, postharvest physiology, and quality outcomes were studied, providing theoretical basis for CA storage of *L. longiflorum* and AI in fruit and vegetable storage.

Material and Methods

Plant materials

L. longiflorum (400 ± 50 g) samples were purchased from a local farmer's market in Yongzhou, Hunan, China, and transported to laboratory in a refrigerated (2–8°C) vehicle.

Materials and instruments

2,6-dichlorophenol indophenol sodium salt (BR; BASF Biological Technology Co. Ltd., Anhui, China), vitamin C (AR; Sinopharm Chemical Reagent Co. Ltd., Shanghai, China), 3,5-dinitrosalicylic acid and Seignette salt (AR; Tianjin Guangfu Fine Chemical Research Institute, Tianjin, China), tetrabutylammonium chloride (AR; Zibo Kangyuan Trade Co. Ltd., Shandong, China), and all other reagents were analytically pure.

A YS-XCAB/62 CA preservation test box (Yishi Technology Co. Ltd., Hangzhou, China), D-8PC UV-visible spectrophotometer (Philes Instrument Co. Ltd., Nanjing, China), 3051H fruit and vegetable respirometer (Top Cloud-Agri Technology Co. Ltd., Zhejiang, China), and CR-400 Chroma Meter (Konica Minolta Holdings Inc., Shanghai, China) were used in this study.

Reagent configuration

Britton-Robinson (BR) buffer solution: 0.04-mol/L acetic acid + 0.04-mol/L phosphoric acid + 0.04-mol/L boric acid were mixed in equal volume. (2) 0.6% tetrabutylammonium chloride buffer solution: 0.6% tetrabutylammonium chloride was added to BR buffer solution.

Terminology explanation

The membership function, also known as the fuzzy meta function, is used in fuzzy sets and indicates the degree of truth that an element belongs to a fuzzy set.

Test design

Pre-treatment of samples

L. longiflorum specimens were chosen with complete scales, free from diseases and insect pests. Specimens were washed and transferred to a CA preservation box on the day of pre-treatment before measurement of relevant indices.

Single-factor testing

The test factors included O_2 (5%, 10%, 15%, 20%) and CO_2 (0%, 4%, 8%, 12%) concentrations, temperature (5°C, 10°C, 15°C, and 20°C), and relative humidity (60%, 75%, 90%). The samples, taken after pre-treatment, were measured for respiration rates and weight loss every 3 days for total of 13 days.

Test design for storage technology optimisation

Based on the single-factor experiment, the RSM test design was combined with GA-NN and used to further optimise parameters for the CA storage of *L. longiflorum* to identify the best CA storage conditions.

Construction of fuzzy mathematical model for changes in the storage quality of *L. longiflorum*

Construction of index set for *L. longiflorum*

The scored value for *L. longiflorum* depends on the overall exterior (e.g., colour and weight) and interior (e.g., taste and nutritional value) qualities. This experiment used a comprehensive score constructed by fuzzy mathematics to evaluate the quality of lily specimens. Index set $\boldsymbol{A}$ is as follows:

$\boldsymbol{A}$ = {A_1: brightness, A_2: weight loss rate, A_3: colchicine, A_4: total sugar, A_5: vitamin C, A_6: flavonoid}

Fuzzy weight vector of *L. longiflorum*

Several experts were invited to give evaluation scores to each index in evaluation index set $\boldsymbol{A}$ based on personal experience. The normalisation method was then used to obtain the required weight matrix $\boldsymbol{a}$.

$$a = [a_1, a_2, \ldots a_6], \tag{1}$$

where a_i is obtained from Equation (2).

$$a_i = \frac{\sum_{m=1}^{x} a_1'}{R}, m = 1,2,\ldots,x, \tag{2}$$

where a_i is the final evaluation of indicator A_i; a_i' is the expert's evaluation of indicator A_i; x is the total number of experts; and R is the sum of evaluations of all indicators.

Calculation of the evaluation index effect of *L. longiflorum*

In the evaluation indices of *L. longiflorum*, brightness, total sugar, colchicine, vitamin C, and flavonoids had positive effects; the membership function was calculated by Equation (3),

$$t(A_n) = \frac{t_n - t_{\min}}{t_{\max} - t_{\min}}. \tag{3}$$

The weight loss rate was negative; the membership function was calculated by Equation (4).

$$t(A_n) = 1 - \frac{t_n - t_{\min}}{t_{\max} - t_{\min}}, \tag{4}$$

where t_n is the evaluation index value of *L. longiflorum* index A_i corresponding to the fuzzy subset, and t_{max} and t_{min} are the maximum and minimum values of *L. longiflorum* index A_i corresponding to the fuzzy subset.

Construction of comprehensive evaluation index of *L. longiflorum*

The vector set $\boldsymbol{E}$ of the comprehensive score e of *L. longiflorum* was obtained according to the selected detection index matrix $\boldsymbol{Q}$ combined with the weight matrix $\boldsymbol{a}$, calculated using Equation (5), where n is the number of subsequent RSM test design groups,

$$E = Q \times a = [e_1, e_2, e_3, \cdots, e_n]. \tag{5}$$

Among them, the detection index matrix $\boldsymbol{Q}$ is the fuzzy matrix calculated by the membership function of

L. longiflorum indices and the number of test groups, as shown below:

$$Q = \begin{bmatrix} Q|A_1 \\ Q|A_2 \\ \vdots|\vdots \\ Q|A_6 \end{bmatrix} = \begin{bmatrix} t_1^1 & t_2^1 & \cdots & t_n^1 \\ t_1^2 & t_2^2 & \cdots & t_n^2 \\ \vdots & \vdots & \ddots & \cdots \\ t_1^6 & t_2^6 & \cdots & t_n^6 \end{bmatrix}.$$

Element t_{ij} in the i-th row and the j-th column in matrix $\boldsymbol{Q}$ represents the membership degree of the fuzzy subset of *L. longiflorum* under the conditions of index A_i corresponding to the j-th column. Since there are six quality evaluation indices in the storage process of *L. longiflorum*, i = 1, 2, 3, 4, 5, 6, j is determined by the number of groups in the subsequent RSM test design and is therefore equivalent to n.

Indicators and measurement methods

Respiration rate

L. longiflorum was placed in fruit and vegetable respirometer. The initial gas composition and proportions were set according to the single-factor test; the test data were collected within 30 min after the CO_2 content became stable. Then, the respiration rate was calculated using Equation (6):

$$C = \frac{\left(a \times \frac{44}{22.4} \times \frac{273}{273+T}\right) - \left(a_0 \times \frac{44}{22.4} \times \frac{273}{273+T_0}\right)}{m \times h} \times \frac{V}{1000}, \quad (6)$$

where C is the respiration rate (mg/[kg h]); a is the CO_2 concentration after measurement (ppm); a_0 is the CO_2 concentration before measurement (ppm); T is the temperature after measurement (°C); T_0 is the temperature before measurement (°C); V is the total volume of the container (dm^3); h is the measurement time (h); and m is the quantity of *L. longiflorum* (kg).

Rate of weight loss

The samples were taken out from CA storage room. After absorbing any external water with absorbent paper, the samples were weighed in electronic balance. The weight of each time point was recorded, and the weight loss rate was calculated by calculating the difference, compared to the initial total weight. The weight loss rate was recorded to two decimal places.

Determination of brightness L*

Luminance (L*) represents the brightness of object's surface. In this experiment, a Chroma Meter CR-400 was used to measure luminance. Before the measurements were recorded, the instrument was calibrated using a standard white tile. Samples were placed on the measuring head of Chroma Meter CR-400, and the brightness of the designated area of test samples was measured.

Total sugar measurement

Total sugar extracts from lily samples were prepared according to the method described by Xiong and Niu (2014) and measured according to the methods described by Zhan *et al.* (2020). Lily polysaccharide extract, 1 mL, was gently mixed with 0.75-mL 6 mol/L HCl solution in a 10-mL volumetric flask. The mixture was boiled in a water bath for 20 min and cooled to room temperature. Then, 1 mL of 6-mol/L NaOH solution and 1.5 mL of 3,5-dinitrosalicylic acid (DNS) reagent were added, and the mixture was boiled in a water bath for 6 min, cooled to room temperature, and water was added to make up a final volume of 10 mL. The absorbance was measured at 550 nm.

Flavonoid measurement

Flavonoid extracts from lily samples were prepared according to the method described by Wang (2016) and measured according to the method reported by Naheed *et al.* (2017). In a 25-mL volumetric flask, 2 mL of lily flavonoid extract, 1 mL of 5% $NaNO_2$, and 1 mL of 10% $AlCl_3$ were added and mixed gently. After 5 min, 4 mL of 20% NaOH was added, and distilled water was added to make up the required volume. After 15 min, the absorbance was measured at 506 nm.

Vitamin C measurement

Content of vitamin C was determined according to the methods used to determine the levels of reductive-form ascorbic acid in foods (GB 5009.86—National Health and Family Planning Commission of PRC, 2016). In a high-speed blender, 5 g of lily bulbs and 20 mL of 5% $H_2C_2O_4$ were added and blended for 15 s, for three times. Then, 4 g of homogenate was transferred to a 10-mL volumetric flask, and the required volume was made up with oxalic acid solution, gently mixed and filtered. The collected filtrate, 5 mL, was placed in a conical flask and titrated with calibrated 2,6-dichloroindophenol solution until the filtrate turned pink and did not fade for 15 s. A blank was performed with oxalic acid in the same way.

Colchicine measurement

Colchicine extracts from lily samples were prepared according to the method described by He (2002) and measured according to the Chinese Pharmacopoeia Part II (Chinese Pharmacopoeia Commission, 2015). In a high-speed blender, 5 g of lily bulbs and 25 mL of anhydrous ethanol were added and blended for 15 s, for three times. After incubation at room temperature for 4 h, the mixture was shaken and filtered. The collected filtrate, 1 mL, was placed in a 10-mL volumetric flask and diluted to the mark with 0.6% tetrabutylammonium chloride BR buffer solution. The absorbance was measured at 320 nm.

Analysis of the influence of RSM test factors on results

All factors other than the one being analysed were kept constant during the analysis of the influence of that factor on RSM test results. Positive effects (a high value of the factor resulting in high test results) were marked as (+), and negative effects (a high value of the factor resulting in low test results) were marked as (-).

Statistical analyses

As most test indicators differed in initial content among multiple *L. longiflorum* specimens, avoiding errors that may result from inconsistent baselines was essential. In addition to selecting *L. longiflorum* with similar indices in the pre-experiment stage, the content before and after storage could also be expressed as a percentage, thus reducing the error caused by different initial contents. In this experiment, total sugar, flavonoids, vitamin C, and colchicine were expressed as percentage values. The respiration rate was only roughly optimised in the single-factor experiment and had a limited impact on the final result, so it was not processed in this manner.

Analysis of variance (ANOVA) was performed on the quality-attributed data to identify significant differences between samples from different storage conditions. Significant changes were identified using Tukey's honest significant difference (HSD) test at the significance level of α = 0.05. Graphs were prepared using Microsoft Excel 2013 and Origin 2018. Design-Expert 8.0.7.1 was used to design RSM tests.

Test Results

Single-factor test results

Effect of O_2 concentration on the respiration and weight loss rates of L. longiflorum

Figure 1A shows change in the respiration rate of *L. longiflorum* over time when the O_2 concentration varied between 5% and 20%. On the fourth day of the test, a significant difference in the respiration rate of O_2 conditions was recorded between 10% and 15%. At the end of the test, the respiration rate at 5% O_2 was 12.01 mg/(kg·h), slightly less than the 13.17 mg/(kg·h) observed at 10% O_2 and significantly less than the 23.88 mg/(kg·h) observed at 15% O_2 and 26.80 mg/(kg·h) observed at 20% O_2.

Figure 1B shows changes in the weight loss rate of *L. longiflorum* over time when the O_2 concentration was between 5% and 20%. The weight loss rate varied between treatment groups on the fourth day of the test. The weight loss rate at 5% O_2 was 3.92%, which was slightly lower (3.95%) than that at 10% O_2 and significantly lower compared to 15% O_2 (4.31%) and 20% O_2 (4.61%).

Based on the single-factor O_2 test results, an O_2 concentration of 5% was selected for the next optimisation step.

Effect of CO_2 concentration on the respiration and weight loss rates of L. longiflorum

Figure 2A shows changes in the respiration rate of *L. longiflorum* over time when the CO_2 concentration varied between 0% and 12%. The CO_2 concentration had a marked effect on the respiration rate of *L. longiflorum*, with the difference observable following the first day only. The respiration rate of *L. longiflorum* at 4% CO_2

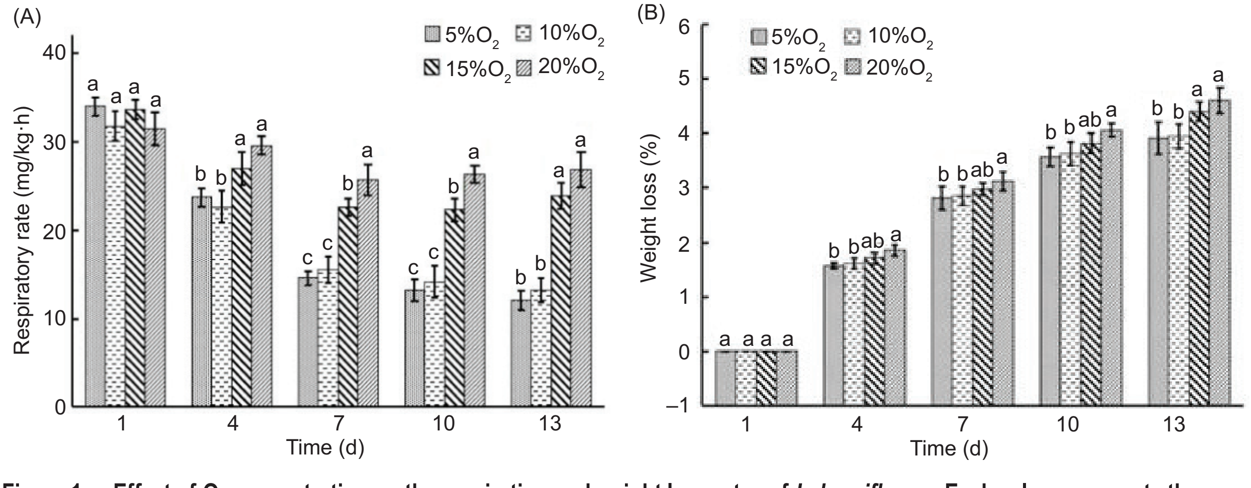

Figure 1. Effect of O_2 concentration on the respiration and weight loss rates of *L. longiflorum*. Each value represents the mean value of three replicates; error bars indicate standard deviation (±SD). Different letters indicate significant differences between different extraction conditions (P < 0.05).

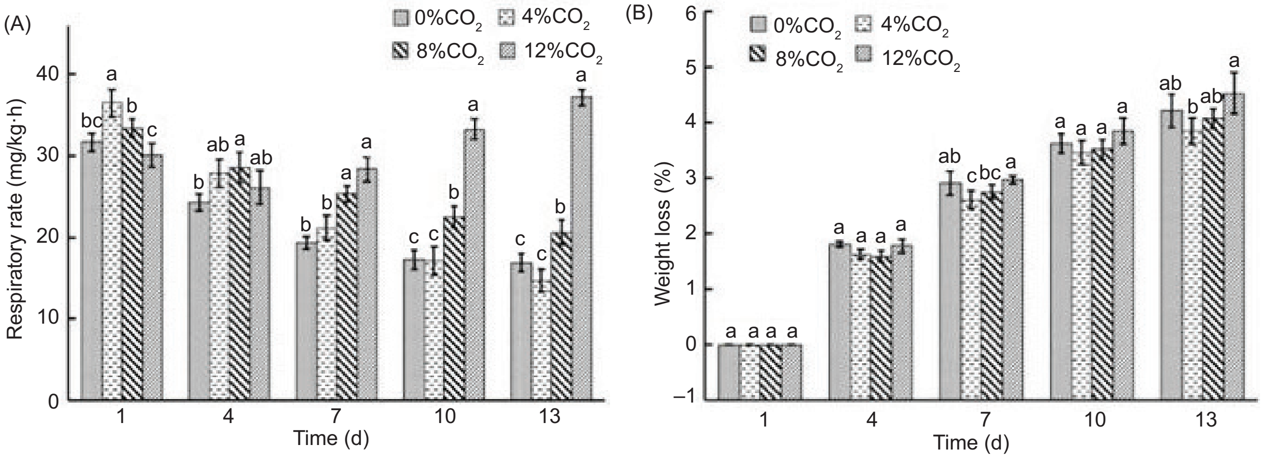

Figure 2. Effect of CO_2 concentration on the respiration and weight loss rates of *L. longiflorum*. Each value represents the mean value of three replicates; error bars indicate standard deviation (±SD). Different letters indicate significant differences between different extraction conditions (*P* < 0.05).

decreased to 14.71 mg/(kg·h), which was lower compared to 16.89 mg/(kg·h) at 0% CO_2 and 20.62 mg/(kg·h) at 8% CO_2. In contrast, the respiration rate at 12% CO_2 did not decrease but increased to 37.15 mg/(kg·h), presumably because fruits and vegetables switch to an anaerobic or respiratory climacteric state at a particular CO_2 concentration (Guo and Cui, 2013).

Figure 2B shows changes in the weight loss rate of *L. longiflorum* over time when the CO_2 concentration was between 0% and 12%. The rate of weight loss of *L. longiflorum* at 4% CO_2 was 3.84%, significantly lower than 4.52% at 12% CO_2 and slightly lower than the weight loss rates of 4.21% at 0% CO_2 and 4.07% at 8% CO_2.

Based on the single-factor CO_2 test results, a CO_2 concentration of 4% was selected for the next optimisation step.

Effect of temperature on the respiration and weight loss rates of *L. longiflorum*

Figure 3A shows changes in the respiration rate of *L. longiflorum* over time when the temperature was between 5°C and 20°C. Different temperatures resulted in different respiration rates of *L. longiflorum* on the first day of the test. By the end of the test, the respiration rate of *L. longiflorum* at 5°C dropped to 10.88 mg/(kg·h), which was significantly lower than the rates of 19.43 mg/(kg·h) at 10°C, 22.09 mg/(kg·h) at 15°C, and 27.97 mg/(kg·h) at 20°C.

Figure 3B shows changes in the weight loss rate of *L. longiflorum* over time when the temperature was between 5°C and 20°C. On the fourth day of the test, the weight loss rate of *L. longiflorum* varied between groups,

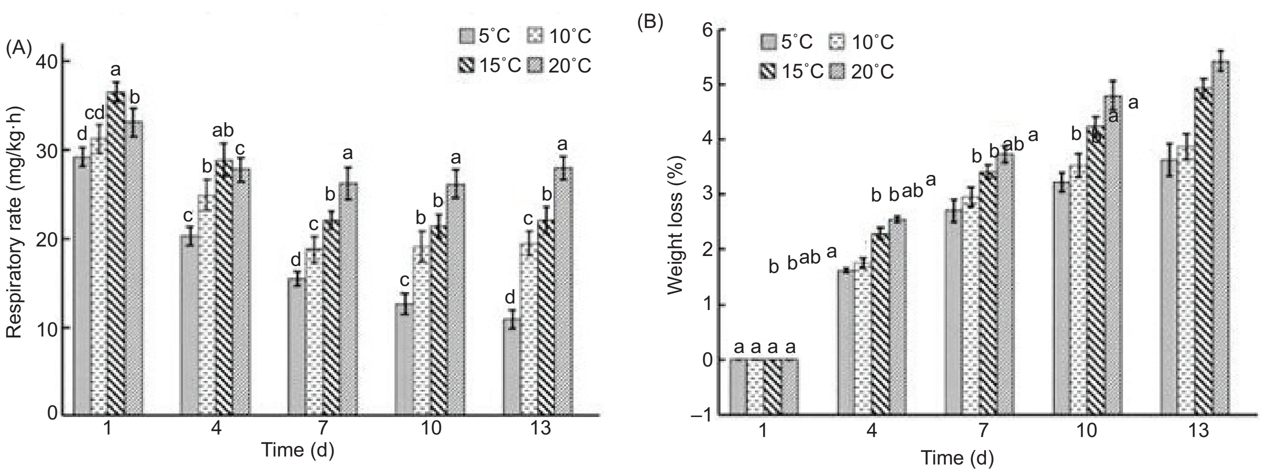

Figure 3. Effect of temperature on respiration and weight loss rates of *L. longiflorum*. Each value represents the mean value of three replicates; error bars indicate standard deviation (±SD). Different letters indicate significant differences between different extraction conditions (*P* < 0.05).

indicating that temperature influenced the weight loss outcome. The rate of weight loss of *L. longiflorum* at 5°C was 3.63%, slightly lower, compared to 10°C (3.87%) and significantly lower than at 15°C (4.93%) and 20°C (5.42%).

Based on the single-factor temperature test results, a temperature of 5°C was selected for the next optimisation test.

Effect of relative humidity on the respiration and weight loss rates of L. longiflorum

Figure 4A shows changes in the respiration rate of *L. longiflorum* over time when the relative humidity was between 60% and 90%. Relative humidity had no obvious effect on the respiration rate of *L. longiflorum* samples. The respiration rate at a relative humidity of 90% was 14.74 mg/(kg·h), slightly lower than 14.94 mg/(kg·h) at 75% relative humidity and 15.12 mg/(kg·h) at 60% relative humidity.

Figure 4B shows changes in the weight loss rate of *L. longiflorum* over time when the relative humidity was between 60% and 90%. Relative humidity significantly affected the weight loss rate of *L. longiflorum* throughout the experiment. The weight loss rate of *L. longiflorum* at 90% relative humidity was 3.14%, significantly lower than the rates of 3.71% at 75% relative humidity and 4.84% at 60% relative humidity.

Based on the single-factor relative humidity test, relative humidity of 90% was selected for the next optimisation test.

RSM test results

Since the upper limit of relative humidity in CA boxes was 90%, and the single-factor test results showed that 90% relative humidity had the best storage effect for *L. longiflorum*, subsequent tests were performed at 90% relative humidity. These tests continued to investigate the effects of temperature, and O_2 and CO_2 concentrations on the storage of *L. longiflorum*.

According to Hu (2005), in the three-factor optimisation test, the RSM designed by the central composite circumscribed (CCC) design produced results close to actual values. Therefore, based on the single-factor test results, the RSM test designed using CCC was combined with GA-NN and used to optimise CA storage parameters for *L. longiflorum*. According to the actual situation, four zero levels and 18 groups of RSM test designs were selected. The relevant information was gathered, and r = 1.414 was obtained (see Table 1 for specific conditions).

L. longiflorum was stored for 20 days under the conditions described in Table 2; the value of each index after storage was divided by its corresponding initial value

Table 1. RSM factor levels.

Level	Factors		
	A Temperature (°C)	B O_2 concentration (%)	C CO_2 concentration (%)
r	10.0	9.0	7.0
1	8.5	7.8	6.1
0	5.0	5.0	4.0
–1	1.5	2.2	1.9
–*r*	0.0	1.0	1.0

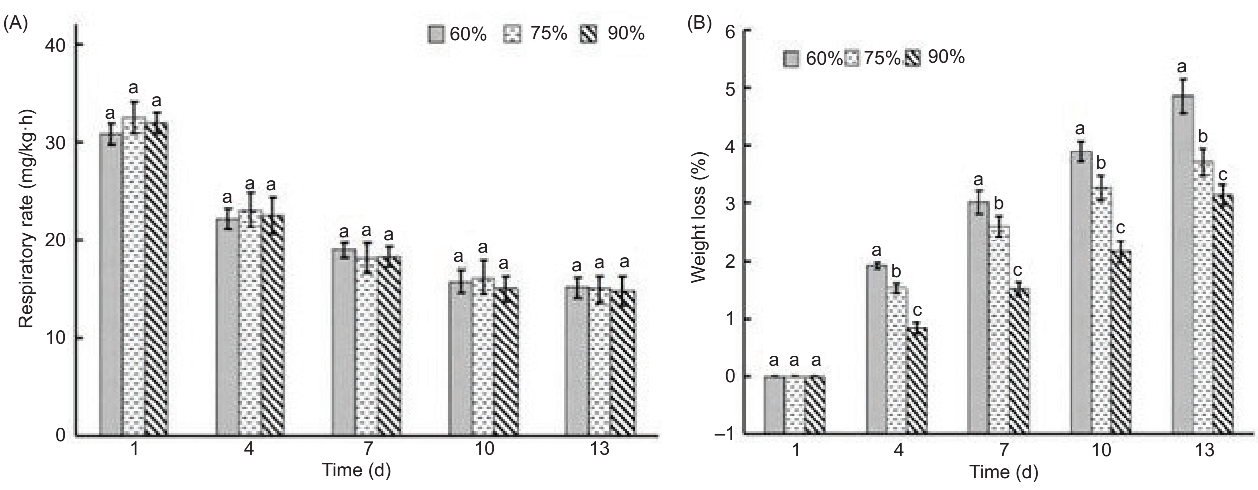

Figure 4. Effect of relative humidity on the respiration and weight loss proportions of *L. longiflorum*. Each value represents the mean value of three replicates; error bars indicate standard deviation (±SD). Different letters indicate significant differences between different extraction conditions (*P* < 0.05).

(0 d) to get the change rate of this index. The results are shown in Table 2.

Index modelling results

Based on personal experience, several experts provided evaluation scores to each index of the evaluation index set ***A*** of *L. longiflorum*. These were combined into an expert evaluation table. The final weightage was calculated using Formula 2, as shown in Table 3.

The total score *R*, calculated as 2165, was the sum of indicator scores provided by all experts. The weight loss rate score was 435, calculated by summing experts' scores. Finally, the weightage of weight loss rate score was calculated as follows; the weights of other indicators could also be calculated according to this method:

Weightage of weight loss rate = 435/2165≈0.201

The comprehensive score e_1 of the first data group in Table 2 was calculated by using Formulae 3–5 and Table 3, and the outcomes are shown below.

$$e_1 = Q \times D = \begin{pmatrix} 0.201 & 0.139 & 0.173 & 0.159 & 0.132 & 0.196 \end{pmatrix} \times \begin{bmatrix} 0.0000 \\ 0.2930 \\ 0.1494 \\ 0.6787 \\ 0.7364 \\ 0.1715 \end{bmatrix} = 0.3053.$$

The number of groups in the RSM test design was 18 (n = 18), so ***Q*** was a 6×18 matrix, and the *L. longiflorum* comprehensive score vector set ***E*** was also composed of 18 groups of comprehensive scores *e*. According to the calculation method of the first group of comprehensive scores e_1, matrix ***E*** was obtained by calculating the remaining 17 groups of comprehensive scores.

Table 2. Change rates for various indices of *L. longiflorum* after 20 days of storage.

No.	A Temp (°C)	B O_2 (%)	C CO_2 (%)	Weight loss rate (%)	Flavon rate (%)	Total sugar rate (%)	Colchicine rate (%)	Vitamin C rate (%)	Brightness rate (%)
1	1	1	1	6.28	45.40	115.06	67.84	47.11	84.93
2	1	1	–1	5.18	41.8	108.60	55.93	41.53	85.52
3	1	–1	1	5.86	57.25	139.39	78.33	52.28	88.68
4	1	–1	–1	4.85	48.98	126.09	77.07	37.81	86.24
5	–1	1	1	4.85	43.56	127.44	59.58	36.36	90.53
6	–1	1	–1	4.50	49.63	121.27	47.88	33.05	88.05
7	–1	–1	1	6.20	44.81	144.77	68.07	51.01	93.48
8	–1	–1	–1	5.11	40.49	133.80	52.16	42.73	87.53
9	*r*	0	0	4.75	55.40	125.97	74.98	54.66	83.16
10	–*r*	0	0	4.25	47.35	149.34	67.06	49.23	92.78
11	0	*r*	0	4.31	54.49	121.21	50.28	26.02	83.82
12	0	–*r*	0	4.33	45.38	129.42	72.69	53.35	92.19
13	0	0	*r*	4.69	40.85	132.13	66.35	52.97	91.61
14	0	0	–*r*	3.95	42.47	118.85	45.68	40.22	84.35
15	0	0	0	3.86	50.70	148.43	69.46	43.39	87.98
16	0	0	0	4.23	54.28	151.61	73.13	43.98	89.26
17	0	0	0	4.18	53.17	147.03	70.14	47.39	89.87
18	0	0	0	4.34	52.37	151.84	73.41	47.85	90.06

Table 3. Expert evaluation form and weightage of experts.

No.	Weight loss rate	Flavone	Total sugar	Colchicine	Vitamin C	Brightness
Expert 1	80	60	70	65	55	80
Expert 2	85	55	75	70	50	90
Expert 3	85	65	80	75	65	80
Expert 4	95	55	75	70	55	85
Expert 5	90	65	75	65	60	90
Weight	0.201	0.139	0.173	0.159	0.132	0.196

$\boldsymbol{E}$ = [0.3053, 0.2685, 0.6819, 0.5249, 0.4749, 0.4103, 0.6074, 0.3896, 0.5949, 0.7823, 0.3651, 0.7148, 0.6145, 0.3390, 0.7324, 0.7890, 0.7784, 0.7994]

Analysis of RSM test results

Based on the test results given in Table 4, each factor in the RSM test designed using CCC had five design groups that met this condition (e.g., the numbers of five groups of temperature factors in Table 2 were: (1,5), (2,6), (3,7) (4,8), (9,10)) according to the method described in analysis of the influence of RSM test factors on results. The results are shown in Table 4.

In Table 4, when the combination of experimental results was [2, 3], the condition was considered to have no noticeable effect on results within the experimental range. A combination of [1, 4] indicated that the factor may have a (+) or (-) effect on the experimental results within the experimental range. In contrast, the combination of [0, 5] indicated that the condition most likely had an effect within the test range. Considering the influence of uncertain factors, such as experimental errors, our results suggested that the factors affected outcomes in the combinations of [1, 4] and [0, 5].

GA-NN Construction and Optimisation

Building BP-NN

(1) *Establishment of virtual samples*
There were 18 groups in the RSM test design, but this was too few for BP-NN. Therefore, virtual samples were introduced to increase the sample size and strengthen network learning ability. The required virtual samples were constructed using an $L_4(2^3)$ orthogonal design table; each group of actual samples produced four groups of virtual samples. Therefore, a total of 90 groups of samples were included. The error range of virtual samples constructed in this experiment was Δi = 0.2% (Zhong *et al*., 2019).

Table 4. Influence of RSM test factors on results.

Factor	Temp	O_2	CO_2
Weight loss rate	3+, 2-	2+, 3-	5+, 0-
Flavone	4+, 1-	3-, 2+	3+, 2-
Total sugar	0+, 5-	0+, 5-	5+, 0-
Colchicine	5+, 0-	0+, 5-	5+, 0-
Vitamin C	4+, 1-	4-, 1+	5+, 0-
Brightness	0+, 5-	4-, 1+	4+, 1-
All	16+, 14-	6+, 24-	27+, 3-
Comprehensive scores	2+, 3-	1+, 4-	5+, 0-

(2) *Determining the number of hidden layer nodes*
We used a three-layer neural network (input layer, hidden layer, and output layer) to create an optimisation model. Temperature, and O_2 and CO_2 concentrations were set as the three-input layer neurons of the network, and the comprehensive score was the output layer node. The number of nodes in the hidden layer was often determined using an empirical formula, such as Equation (7). According to the empirical formula, the number of hidden layer nodes was selected from 3 to 13. The mean square error of the test set was used to evaluate the BP-NN model's accuracy. Using this method, the number of hidden layer nodes was determined to be 10,

$$h = \sqrt{m+n} + a, \tag{7}$$

where m is the number of nodes in the input layer; n is the number of nodes in the output layer; and a is the empirical constant, usually taken from 0 to 10.

(3) *Neural network model evaluation*
After determining the number of hidden layer nodes, the "3-10-1" neural network structure was used to establish a relevant optimisation model. When training the network, the training target error was 0.00001, the learning rate was 0.1, the momentum constant was 0.9, and the maximum number of training steps was 10,000. Next, the obtained comprehensive evaluation and virtual samples were put into the constructed BP-NN, and the resultant evaluation graph is shown in Figure 5. R^2 = 0.9964 indicated that BP-NN performed well and could be used for GA optimisation.

Optimisation of Genetic Algorithm

Relevant parameters of GA were set as follows: population number, 50, maximum genetic generation, 100, cross selection, 0.4, and mutation rate, 0.2. Then the trained neural network model was loaded, with the comprehensive score of *L. longiflorum* used as the output value. The global optimisation of CA process was undertaken, resulting in the fitness curve of the comprehensive scores of *L. longiflorum* as shown in Figure 6. Following 16 iterations, the optimal comprehensive score of *L. longiflorum* was 0.8372. Meanwhile, the following predicted test conditions were included: temperature 4.24°C, O_2 concentration 4.51%, and CO_2 concentration 3.78%.

Verification experiment

Model validation

The results obtained by GA-NN optimisation, combined with real-world data, resulted in the following ideal

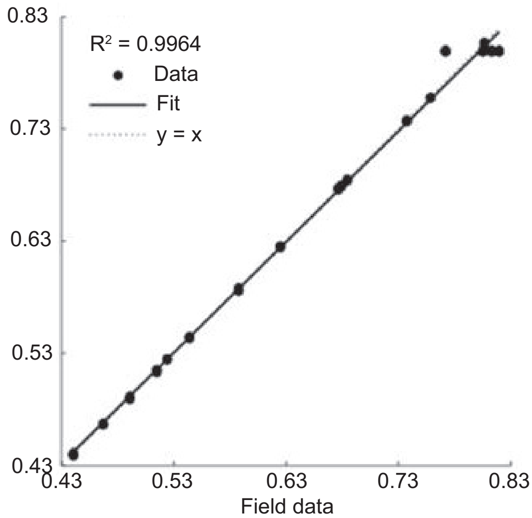

Figure 5. The neural network evaluation chart.

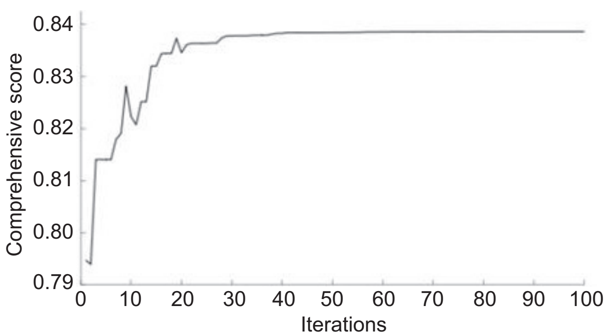

Figure 6. Fitness curve for the comprehensive score of *L. longiflorum*.

parameters: storage temperature 4.2°C, O_2 concentration 4.5%, and CO_2 concentration 3.8%. *L. longiflorum* samples were placed under these storage conditions for 20 days. The changed proportions of all indexes of *L. longiflorum* were as follows: weight loss rate 3.46%, flavone 51.84%, total sugar 151.49%, colchicine 68.99%, vitamin C 46.66%, and brightness 91.96%; the calculated comprehensive score of 0.8424 was obtained. This score was close to the predicted value of 0.8372, indicating that the model was valid.

Verification of storage test

Lilies were placed in either 4°C cold storage or optimised CA storage. Relevant indicators were measured according to the test requirements and the comprehensive score was determined. Changes in the final comprehensive scores are shown in Figure 7. The overall outcome for the lilies stored in CA storage was better than that stored at 4°C cold storage for both storage time and ability to alleviate quality degradation.

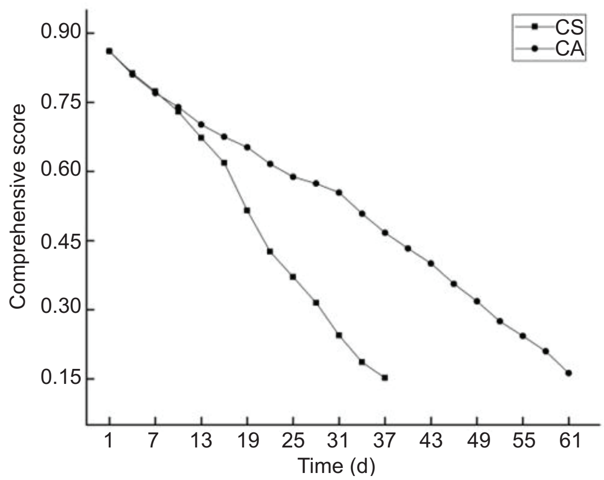

Figure 7. The comprehensive scores of samples over 20 days, comparing refrigerated and modified atmosphere storage results at 4°C.

Discussion

After harvest, physiological activities of fruits and vegetables are mainly based on respiration and transpiration proportions. These physiological activities are affected by their current state and external environmental factors (Ji, 2016). In this experiment, O_2 and CO_2 concentrations were studied as respiration substrates, along with temperature and relative humidity as external factors affecting the metabolism of fruits and vegetables. Therefore, respiration and weight loss proportions were preliminary indicators for optimisation test and development.

In order to more accurately describe the state of *L. longiflorum*, an evaluation index set comprising multiple dimensions was required (Guo and Cui, 2013). Therefore, BP-NN optimisation was used to develop an evaluation index that considered the appearance (colour, brightness, and weight and weight loss rate) and internal indicators (flavour, total sugar, colchicine, nutritional value: vitamin C, and flavonoid content) combined into a single and comprehensive score.

Fruits and vegetables undergo both aerobic and anaerobic respiration. Different respiration patterns can occur depending on the external environment (e.g., O_2 and CO_2 concentrations). Previous studies (Du *et al.*, 2021; Martins *et al.*,2014; Wei *et al.*, 2020) have reported that O_2 concentration is positively correlated with the respiration rate of fruits and vegetables with conventional O_2 concentration because O_2 acts as a substrate for respiration. However, lower O_2 concentration inhibits cytochrome-C oxidase in electron transport chain (Gupta *et al.*, 2009), thereby inhibiting respiration (Figure 1). Furthermore, reducing the concentration of environmental O_2 is favourable for maintaining fruit and vegetable weight, which is consistent with the experimental results of Gao *et al.* (2020). This could be due to decreased respiration rate, leading to decreased dry matter consumed by *L. longiflorum* and decreased respiration heat. Decreasing respiration heat slows down water loss and reduces the rate of weight loss.

Experimental results depicted in Figure 2 show that an appropriate increase in CO_2 concentration inhibits respiration rate because CO_2 is a product of respiration and can interfere with the Krebs cycle enzymes at high concentrations, leading to decreased fruit metabolism (Martins *et al.*, 2014). However, excess CO_2 induces anaerobic respiration (Poonsri, 2021; Junior *et al.*, 2019), increasing the proportion of CO_2 release from fruits and vegetables. Moreover, because the energy conversion efficiency of anaerobic respiration is low, and large quantities of harmful substances are produced, this inevitably results in dry matter loss and increased cell inactivation, a decrease in water storage capacity, and an increase in the final weight loss rate.

Temperature influences the kinetic energy of molecules, thus affecting the rate of chemical reaction. Results (Figure 3) of the study show that higher temperatures in the tested temperature range increased the rate of respiration and weight loss. Biological enzyme and molecular activities increase with increasing temperature (Pan *et al.*, 2009). Higher levels of enzymatic and molecular activities cause greater dry matter consumption in fruits and vegetables. Additionally, active water molecules bound by the tissues of fruits and vegetables decrease, allowing them to reach more easily the surface of *L. longiflorum* and evaporate to the storage environment (Li, 2017).

Environmental humidity forms a humidity gradient difference with *L. longiflorum.* This results in loss of internal water in *L. longiflorum* through a gradient. The speed of water loss is directly related to the humidity gradient difference; so, higher environmental humidity results in lower humidity gradient differences between *L. longiflorum* and the storage environment and slower water loss. Consequently, loss of weight loss is lower when the environmental humidity is higher (Figure 4B). In addition, in a more humid external environment, heat is easily transmitted to the environment through thermal convection, thus reducing the respiratory heat accumulated in fruits and vegetables and inhibiting respiration. However, when the ambient temperature is low, the heat released by respiration decreases, resulting in less accumulated heat, which may be the reason for insignificant difference in respiration rates (Figure 4A). In summation, appropriately reducing the temperature and O_2 concentration and increasing the relative humidity and CO_2 concentration inhibit the respiration rate of *L. longiflorum* (Banda *et al.*, 2015; Belay *et al.*, 2017; Maree *et al.*, 2022), thus achieving a better preservation effect for fresh *L. longiflorum*.

The data given in Table 4 show that the weight loss rate of *L. longiflorum* is correlated with the CO_2 concentration of RSM test. However, it also correlates with temperature and O_2 and CO_2 concentrations in single-factor test. This may be because, within the range of RSM test, interaction between temperature and O_2 concentration substantially affected the weight loss rate of lily samples.

Change in total sugar content was due to low-temperature saccharification of *L. longiflorum* during dormancy, a phenomenon where lower temperatures increase the sugar content of fruits (Kang *et al.*, 2020; Langhans and Miller, 1990). The total sugar levels are negatively correlated with O_2 and positively correlated with CO_2, perhaps because a low-O_2 and high-CO_2 environment inhibits respiration, thereby reducing sugar consumption.

Variations in flavonoids, vitamin C, and colchicine levels are similarly affected by experimental factors, possibly because flavonoids, vitamin C, and colchicine are secondary metabolites of plants (Gong *et al.*, 2011; Zhang *et al.*, 2022). Among them, change in flavonoid content was positively correlated with temperature, consistent with the changes in flavonoid content in the storage test of lilies in other experiments (Wei *et al.*, 2021). Gong *et al.* (2011) found that low temperature is not conducive for vitamin C preservation during storage of lily. A proper increase in temperature reduces vitamin C loss. Thammawong *et al.* (2019) found that lowering O_2 concentration is beneficial to preserve vitamin C in fruits and vegetables; simultaneously, a sufficiently high temperature also reduces the loss of vitamin C in a low-O_2 environment. Yang *et al.* (2015) treated fruits and vegetables with a *polyethylene* (PE) film, increasing the concentration of CO_2 in the environment, showing that a measured increase in CO_2 is beneficial for preserving vitamin C.

An evident change in colchicine content in fruits and vegetables under different storage conditions was not found. However, as a secondary metabolite of lily, colchicine is expected to reflect changes in the overall secondary metabolite levels. Both O_2 and CO_2 reduce the metabolism of fruits and vegetables by affecting respiration, thus reducing the consumption of secondary metabolites.

Vincenzo *et al.* (2020) reported that an appropriate increase in temperature would benefit the synthesis of secondary metabolites. Although polyphenol oxidase (PPO) and polyphenol peroxidase (POD) enzymatic activities would also increase, the synthesis of secondary metabolites was greater than the consumption, so it increased overall (Zhao *et al.*, 2021). The elevated PPO and POD enzymatic activities are also expected to affect the brightness of fruits and vegetables, because browning of fruits and vegetables includes enzymatic and non-enzymatic browning. Enzymatic browning is mainly caused by the conversion of polyphenols into quinones by PPO and POD enzymes in the presence of H_2O_2 to form brown substances. Non-enzymatic browning occurs due to the dehydration and condensation of amino- and carbon-based compounds to form brown pigments.

Both enzymatic and non-enzymatic browning reduces the brightness of fruits and vegetables (Nooshkam *et al.*, 2019). In the surveyed temperature range, enzyme activity and respiration rates (Figure 3A) were positively correlated with temperature, while generated H_2O_2 levels and respiration rates were also correlated positively. Increasing temperature aggravates enzymatic browning. In addition, carbon-based compounds in non-enzymatic browning are primarily reducing sugars (Cha *et al.*, 2019). At higher temperatures, the content of reducing sugars in lily further increases and aggravates non-enzymatic browning (Zhao *et al.*, 2021). Therefore, there is a negative correlation between temperature and lily brightness. Moreover, Du *et al.* (2021) and other studies reported that fruits and vegetables are more prone to browning when O_2 is high, while appropriately increase in CO_2 effectively inhibits the browning of fruits and vegetables (Pace *et al.*, 2020). Such a relationship occurs because O_2 and CO_2 affect browning by affecting respiration rate. However, concentrations of O_2 and CO_2 need to be maintained at levels that do not produce anaerobic respiration, which leads to the accumulation of ethanol and acetaldehyde, increases electrolyte leakage, increases total phenol content, and accelerates the browning reaction (Wei *et al.*, 2020).

Generally, within the range of factors in this RSM experiment, the effect of temperature on the outcomes was most complicated, likely because the interaction between temperature and other factors was more evident. O_2 concentration negatively affected the results, and CO_2 concentration had a positive effect, consistent with the comprehensive evaluation results. Such alignment indicates that comprehensive evaluation modelling has high credibility and practical application value.

In the optimisation process, this paper adopted GA-NN, which had a fitting advantage over RSM (Bhatti *et al.*, 2011; Dong *et al.*, 2017; Mitra *et al.*, 2019). Virtual samples were introduced to solve the training problems that were caused by low sample size. An excellent fitting effect was obtained (R^2 = 0.9964), and the final comprehensive evaluation result of the prediction was 0.8372, very close to the verification result of 0.8424, indicating that the GA-NN optimisation outcome was reliable. The optimised CA storage conditions and 4°C cold storage temperature were also tested, and the comprehensive score changes were compared. CA storage alleviated the decreasing comprehensive score of *L. longiflorum* and prolonged its acceptable storage period. Therefore, the optimised parameters presented herein could be used for investigating the CA storage of *L. longiflorum*.

Conclusion

Based on single-factor results, GA-NN was used to optimise the storage conditions of *L. longiflorum* in CA. The results showed that storage of *L. longiflorum* in CA at a storage temperature of 4.2°C, O_2 concentration of 4.5%, CO_2 concentration of 3.8%, and humidity of 90% preserved the quality of lilies for a longer period compared with 4°C (Figure 7). Subsequently, CA storage replaced 4°C cold storage for collecting *L. longiflorum*. BP-NN belongs to the black-box model, making understanding the relationship between independent and dependent variables challenging. Therefore, the positive (negative) effects of independent variables on dependent variables were evaluated. Based on this, a simple analysis was undertaken that led to several conclusions. It is worth mentioning that the judgement standard of fruit and vegetable quality relies on different factors. Variables affecting these factors are also complex, forming a multi-level network structure with quality as a primary outcome. This is similar to the structure of BP-NN, with the two having the same logical basis. Such an overlapping relationship infers that AI has a deeper association with fruit and vegetable storage and can be used for different research scenarios in the future.

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Conflict of interest

No competing financial interests were declared by authors.

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