

1. Introduction

1.1 Background and Motivation

As cut flowers are primarily traded for special occasions, it is important to maintain their ornamental value for a long time. Accordingly, research has been conducted on preservation methods to extend the vase life of cut flowers, and methods that the public can easily follow have also become known.

Although various methods for maintaining floral freshness have been reported in the literature, these guidelines are often generalized rather than tailored to specific floral species. However, each flower possesses distinct physiological characteristics, including variations in water absorption and temperature sensitivity. Given that roses (*Rosa hybrida*) are among the most predominant cultivars utilized in commercial bouquets, establishing optimized protocols for this specific genus is highly warranted. Therefore, this study investigates the effects of commonly available preservation environments on maintaining the freshness of roses.

1.2 Research Question

Among commonly used preservation methods, this study focuses on how the type of water affects the vase life of roses. The experiment aims to identify the type of water that can most effectively preserve roses among the types of water used for flower preservation.

2. Experimental Design

2.1 Overview

This study employs a 2×2 factorial design to investigate the effects of water type on the vase life of cut roses. Two factors are considered : Sugar(With or Without) and Vinegar(With or Without), which leads to four treatments. The experiment is conducted across two locations, with 8 replications per treatment. The response variable is freshness of cut roses, measured twice-daily.

2.2 Definition of Treatments

Tap water, 2% Sugar water, 0.6% White Vinegar water, 2% Sugar + 0.6% White Vinegar water is used in the experiment. To prevent differences between the initial and subsequent water temperature, each treatment is kept at room temperature for 30 minutes before use. To make the experiment easy to conduct at home, the most readily available Beksul White Sugar and OTOKI White Vinegar are used for this experiment. White Vinegar is a colorless, transparent vinegar made using distilled spirits produced by fermenting grains. Unlike regular vinegar, it contains no sugar. Therefore, it was selected to examine only the effects of pure vinegar.

The first treatment is tap water, which serves as the control in this experiment. It is defined as 100ml of tap water left at room temperature for 30 minutes.

The second treatment is sugar water, prepared at a concentration of 2%. 2g of sugar is dissolved in tap water, and the final volume is adjusted to 100mL. After dissolution, the solution is left at room temperature for 30 minutes. The 2% concentration is adopted from *De Silva et al.* (2013)

The third treatment is white vinegar water, prepared at a concentration of 0.6%. As measuring 0.6 mL directly poses practical difficulties, 4.8 mL of white vinegar is added to tap water and the final volume adjusted to 800 mL, which is then divided into 100 mL samples for use. The solution is left at room temperature for 30 minutes. The 0.6% concentration is adopted from *De Silva et al.* (2013)

The fourth treatment is a solution of 2% sugar and 0.6% white vinegar. 4.8 mL of white vinegar and 16 g of sugar is added to tap water, and the final volume is adjusted to 800 mL. This solution is then divided into 100 mL samples for use. The solution is left at room temperature for 30 minutes. The treatment concentration is adopted from *De Silva et al.* (2013)

To minimize the risk of microbial contamination, each treatment is replaced at 48-hour intervals throughout the experimental period.

2.3 Definition of Response Variable

The response variable is rose freshness which is defined as the number of days a cut rose stem remains its initial condition.

2.3.1 Termination Criteria : VBN Rosa Evaluation Card

Termination criteria were established using the VBN Rosa Evaluation Card (FloraHolland, 2014), which was developed by VBN (Vereniging van Bloemenveilingen in Nederland), the leading Dutch flower auction association and a widely recognised authority in postharvest quality standards for cut flowers. This card provides standardised evaluation criteria developed specifically for cut roses.

The VBN card encompasses a wide range of criteria, but applying all of them was not practical given the time constraints and the risk of subjective judgment. Six criteria were therefore chosen: those with clear, observable write-off conditions to minimise ambiguity, and those covering different parts of the flower so that both floral and foliar deterioration could be considered.

Table 2.1 *Flower-Related Termination Criteria*

Criterion	Description	Write-off
Bent Neck	Drying and kinking of the stem just below the bud	Flower angle > 90°
Brown Flower	Petals clearly turning brown, not due to Botrytis	> 5 petals clearly (more than half) brown
End of Flowering	Rose fully opened (stage 4–5), petals falling and/or discoloring	> 2 petals fallen, or flower visibly limp (<i>discoloration alone NOT sufficient</i>)

Bent neck was selected as criterion because it represents a well-recognised indicator of vascular deterioration in cut roses (Daneshmand et al., 2024).

Brown Flower and End of Flowering were additionally included as both target distinct aspects of flower deterioration and provide relatively clear and observable indicators for visual assessment.

Table 2.2 *Stem-Related Termination Criteria*

Criterion	Description	Write-off
Kinking	The stem kinks	Upon detection
Botrytis	The stem discolours gray to black, sometimes gray fluffy mold appears, the part of the above the affected area withers and the flower withers.	Upon detection

For stem-related criteria, both kinking and botrytis were included. Kinking was selected as a direct indicator of stem condition. Botrytis was retained as one of the most frequently occurring fungal pathogens in cut roses (Muñoz et al., 2019)

Table 2.3 *Foliage-Related Termination Criteria*

Criterion	Description	Write-off
Leaf Shedding (Abscission)	Leaves fall from the stem	> 50% of total leaves fallen
Yellow and/or Brown Foliage	Leaves turn yellow and/or brown	> 50% of total leaf surface area or > 50% of total leaf count

Two foliage-related criteria were selected to ensure a comprehensive evaluation of foliage condition. Specifically, Leaf Shedding captures physical loss, while Yellow and/or Brown Foliage accounts for visual deterioration, together covering both quantitative and qualitative aspects of foliage condition.

2.3.2 Measurement Method

Observations are conducted twice daily at 08:00 and 20:00, providing a consistent 12-hour interval. Freshness is recorded in 0.5-day increments for each session. Visual scoring follows the termination criteria specified in Section 2.3.2. Each vase is assessed as a single experimental unit.

All observers use a standardised A4 white paper sheet as a shared colour reference background during assessment. All observations are conducted under artificial lighting to eliminate variability caused by natural light fluctuations throughout the day.

Environmental conditions, specifically temperature and humidity, are monitored throughout the experiment. Both factors are known to influence postharvest quality and the rate of floral deterioration in cut roses (Daneshmand et al., 2024).

Visual logs are recorded photographically at each observation session using a smartphone mounted on a fixed tripod at a consistent position. To maximise colour accuracy and reproducibility, the camera is set to manual mode with the following fixed parameters.

- ISO 50 : To minimise image noise
- White balance 5500K : To approximate natural daylight and ensure colour consistency across sessions
- Shutter speed 1/100s : To eliminate flickering artefacts from artificial lighting
- In-built software colour correction disabled : To prevent automatic adjustments from altering colour representation between sessions

2.3.3 Supplementary Measurement : ImageJ

Relying on visual observation alone has a risk of subjectivity, especially when evaluating colouration, as subtle colour changes can be perceived differently by observers. Therefore, ImageJ, a widely-used open-source image analysis software for quantifying colour changes and morphological features, is employed as a supplementary measurement tool. (ImageJ, 2007)

Captured visual logs will first be colour-calibrated using an A4 white sheet of paper as a reference, ensuring consistent colour measurement despite variations in lighting across sessions and locations. After calibration, ImageJ will be used to extract quantitative colour values from the petal regions in each image. This process is expected to improve the precision of discolouration assessment.

2.4 Experimental Units and Replication

2.4.1 Experimental Units

The experimental unit is a single cut rose placed in a vase. Each vase is treated and observed independently throughout the experiment.

2.4.2 Replications

A priori power analysis was conducted based on the standard deviation obtained from De Silva et al. (2013). Under power = 0.8 and $\alpha = 0.05$, the calculation was performed by adjusting for potential variability with a standard deviation of $4 \times SD$, yielding $n = 7.388$. Under replication = 8, Cohen's $f = 0.656$, and $\alpha = 0.05$, the calculated power was 0.839, which is greater than 0.8, so replication = 8 was finally adopted. Full details of the power analysis are provided in Section 5.

2.5 Blocking and Randomisation

2.5.1 Blocking by Location

The experiment is conducted across two separate locations given the limited space available. At each location, 4 replications are conducted for every treatment combination, so that location-related variability including natural light exposure, air circulation, or other site-specific conditions do not affect treatment estimates.

2.5.2 Randomisation Procedure

Each experimental unit is a single rose, occupying one A4 sheet of paper as its designated space. Prior to the experiment, all available spaces within the experimental area are numbered sequentially, each corresponding to one A4 sheet. Treatment assignments are then randomised in R using a fixed random seed (`set.seed([99])`) to ensure reproducibility.

2.6 Environmental Control

Previous studies have maintained strict environmental control during postharvest experiments, including constant temperature and uniform light intensity (De Silva et al., 2013). In the present study, however, complete environmental control is not feasible given the home setting. Therefore, temperature and humidity will be monitored and recorded with a digital thermo-hygrometer at 08:00 and 20:00 each day.

The experiment will be conducted indoors at an ambient temperature of approximately 23–25°C, which is higher than the 20°C maintained by De Silva et al. (2013). Although higher ambient temperatures accelerate bacterial proliferation in vase solutions (Van Doorn et al., 2013), frequent replacement of sugar-based solutions may inadvertently promote microbial growth by continuously supplying fresh nutrients (De Silva et al., 2013). Balancing these two considerations is essential; therefore, vase solutions will be replaced every two days.

3. Justification for Design Choices

3.1 Justification for Factors and Levels

3.1.1 The reason for setting water type as a factor

Methods used to extend the vase life of cut flowers and maintain freshness include cold storage and wet storage with cut flower preservative (KATI, 2007). Since cold storage is a method used to maintain freshness during distribution, it was considered appropriate to discuss wet storage with cut flower preservatives rather than cold storage.

3.1.2 Reasons for choosing 4 types of water (tap, distilled, sugar, vinegar)

Sucrose, 8-HQS (8-hydroxyquinoline sulfate), STS (silver thiosulfate), organic acids, and antioxidants are commonly used to extend the vase life and maintain the freshness of cut flowers (KATI, 2007). Sugar acts as respiratory substrates to delay aging, increase body weight, and facilitate water absorption by raising osmotic pressure. Aluminum sulfate, HQC, HQS, etc., exert a bactericidal effect and facilitate water absorption by acidifying the preservative solution (Kim, 2001). Therefore, among readily available items in daily life, white sugar was selected from sugars that delay aging, and white vinegar was selected from acidic substances that acidify the preservative solution. Sugar and vinegar have also been used in previous studies (De Silva et al., 2013).

3.1.3 Reason for selecting the subject of observation (rose) and Significance of the study

In the survey of cut flower distribution in the private market from May to October 2022, rose sales outlets were the most numerous, except for May (Lee et al., 2022). This suggests that roses are the most easily accessible flowers for consumers. As roses are among the most commonly purchased cut flowers, findings from this study are directly applicable to everyday consumer settings.

3.2 Justification for Response Variable

3.2.1 Reason for selecting Vase life as the response variable

Although quality evaluation in the cut flower industry is generally based on external characteristics such as color and shape (In et al., 2016), vase life is perceived as an important quality indicator that influences marketability and consumer satisfaction (Sutrisno et al., 2025). In other words, vase life is not only one of the most important factors influencing customer satisfaction, but also a significant factor in repurchase (Vehniwal & Abbey, 2019). Therefore, this study investigates methods to sustain the ornamental value of roses by focusing on vase life rather than external grading factors.

3.2.2 Reason for measuring in 12-hour increments

In this experiment, changes in the quality of cut roses are observed at 12-hour intervals. This interval was established considering the characteristics of roses, which undergo rapid physiological changes after harvest (KATI, 2007). Observations at 12-hour intervals are commonly observed in cut flower research. (Sui et al., 2015)

3.3 Justification for Termination Criteria

VCN card provides standardised evaluation criteria developed specifically for cut roses. Furthermore, the Netherlands accounts for 60% of the global flower market (KATI, 2023). As VCN is the higher-level organization overseeing Dutch cooperative flower auctions, VCN standards can be considered a globally reliable indicator.

4. Power Analysis

According to De Silva et al. (2013), Extending the Vase Life of Gerbera (Gerbera hybrida) Cut Flowers Using Chemical Preservative Solutions, the average vase lives for distilled water (T2), vinegar treatment (T3), and sugar with vinegar treatment (T5) were 10.67, 12.67, and 14.33 days, respectively (SD = 0.57).

As data for all four treatment conditions were not available from a single source, the effect size was estimated from the three most comparable treatments reported by De Silva et al. (2013). This study, however, differs from the present experiment in two respects: it was conducted on Gerbera hybrida rather than Rosa hybrida, and the solutions were prepared using distilled water rather than tap water. In the absence of a more directly comparable source, De Silva et al. (2013) was selected as the closest available approximation.

To estimate Cohen's f from these three group means, the grand mean was first calculated as $(10.67 + 12.67 + 14.33) / 3 = 12.557$. The standard deviation of the group means was then computed as $\sqrt{[(10.67 - 12.557)^2 + (12.67 - 12.557)^2 + (14.33 - 12.557)^2] / 3} = 1.496$. Dividing by the pooled SD yields Cohen's $f = 1.496 / 0.57 = 2.625$, indicating a large effect of water treatment type on vase life. This f value is used as an estimate of the effect size that water type is expected to have on vase life, applied to the present study's 2^2 factorial structure ($k = 4$) for the purpose of power calculation.

Unlike De Silva et al. (2013), which was conducted in a fully controlled environment, the present study is designed to assess the effects of water type in typical daily life conditions. Because complete environmental control is not feasible, greater error variance and an increased standard deviation are anticipated. Four scenarios were therefore constructed by incrementally increasing the assumed standard deviation to reflect possible increases in error variance under less controlled conditions.

Table 4.1 Standard Deviation Scenarios and Corresponding Power at $n = 8$

Scenario	SD	σ^2	Cohen's (f)	Replications	Power at $n=8$
Original SD	0.57	0.325	2.625	-	~1.00
2× SD	1.14	1.300	1.312	3	~1.00
3× SD	1.71	2.924	0.875	5	0.9818
4× SD	2.28	5.198	0.656	8	0.8390

Based on this analysis, $n = 8$ replications were selected, corresponding to an experimental design of at two locations, with 4 replications per at each location. To maintain an equal number of replications across locations, an even number of replications was required; 8 was therefore adopted as the most practical value. Even under the most conservative scenario (4×SD), $n = 8$ replications are expected to yield a statistical power of $1 - \beta = 0.839$ at $\alpha = 0.05$, exceeding the conventional threshold of 0.80.

5. Analysis Plan

5.1 Response Variable and Data Type

The response variable, vase life, is measured in 0.5-day increments. Although the measurements are technically discrete, the number of possible values is sufficient to approximate a continuous scale for analysis purposes. Two-way ANOVA will be used to compare vase life across treatment groups, consistent with pilot studies in postharvest vase life research.

5.2 Exploratory Data Analysis

Before the formal analysis, exploratory data analysis will be conducted to examine the distribution of the data. Boxplots for each treatment group will show how vase life is distributed across treatments. An interaction plot of cell means will also be constructed to provide a visual indication of whether the two factors interact. Basic summary statistics — the mean and standard deviation — will also be calculated for each treatment group and block.

5.3 Statistical Model

As this study employs a 2×2 factorial design with two-way ANOVA the following model is fitted:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + \varepsilon_{ijkl}$$

- μ : the overall mean
- α_i : Main effect of Sugar, $i = 1$: Without Sugar, $i = 2$ With Sugar
- β_j : Main effect of Vinegar, $j = 1$: Without Vinegar, $j = 2$ With Vinegar
- $\alpha\beta_{ij}$: The interaction between the two factors
- γ_k : The block effect of location, $k = 1, 2$
- ε_{ijkl} : The random error term, assumed to be independently and identically distributed as $N(0, \sigma^2)$.

As mentioned in 2.5.1, location is included as a fixed blocking factor in the model.

5.4 Assumption Checking

Three assumptions underlying will be examined. First, independence of errors is assumed to be satisfied through randomisation. This will be verified using an index plot of residuals. Second, normality of residuals will be observed through a normal probability plot (NPP). Non-normality will be detected when points stray from the reference line. Lastly, constant variance will be examined using a plot of residuals versus fitted values. A random scatter around zero indicates that the assumption holds. If violations are detected, a log or Box-Cox transformation will be applied to the response variable prior to analysis.

5.5 Hypotheses

Three hypotheses are tested through this experiment:

- $H_0: \alpha_1 = \alpha_2 = 0$ (Addition of Sugar has no effect on vase life) $H_1: \alpha_i \neq 0$ for at least one i
- $H_0: \beta_1 = \beta_2 = 0$ (Addition of Vinegar has no effect on vase life) $H_1: \beta_j \neq 0$ for at least one j
- $H_0: (\alpha\beta)_{ij} = 0$ for all i, j (no interaction between Sugar and Vinegar) $H_1: (\alpha\beta)_{ij} \neq 0$ for at least one i, j

The interaction term is tested first. If significant, main effects will not be interpreted independently. Instead, simple effects analysis will be conducted to examine the main effect.

5.6 Analysis of Variance

A two-way ANOVA will be conducted to test the stated hypotheses. The ANOVA table will partition the total variation into the following sources: Factor A (Addition of Sugar), Factor B (Addition of Vinegar), the A×B interaction, Block (location), and residual error. Each effect is tested using an F-statistic at a significance level of $\alpha = 0.05$. A p-value below 0.05 leads to rejection of the corresponding null hypothesis.

As blocks simply exist, no inference will be made about the block effect (Oehlert, 2010). It is further assumed that treatment effects are consistent across blocks — that is, no block-by-treatment interaction is present.

5.7 Post-hoc Analysis

If the interaction term is significant, Tukey's HSD will be applied to all four cells to identify which treatment combinations differ significantly. If the interaction is not significant, main effects will be interpreted independently. As each factor has only two levels, no further comparison is required for main effects.

6. Modifications from Proposal

6.1 Simplification of Factors

The initial experimental design included the stem-cutting method as one of the factors. However, a review of the literature indicated that research on the relationship between cutting method and vase life in cut roses is limited. Moreover, several studies have shown that water type has a more substantial and consistent influence on vase life. Therefore, the experimental design was modified to a 2×2 factorial structure, focusing solely on the effects of water type on the vase life of cut roses.

6.2 Refinement of Response Variable

The initial response variable was assessed using four criteria: petal discolouration, petal abscission, stem bend, and leaf chlorosis. However, the previous design applied relatively few standards to petal evaluation. To enable a more precise and comprehensive assessment, the criteria were expanded and specified. Specifically, bent necks were added to the flower category, Botrytis was added to the stem category, and leaf shedding was added to the foliage category.

In addition, the scoring method was revised. Whereas the original design used a summative average approach, the updated protocol follows the VBN standard, which terminates a rose if any single criterion is breached. This ensures that freshness is judged more strictly and consistently.

Moreover, reducing the number of roses per vase to one allowed for more replications and made individual evaluations clearer and more manageable. This method aligns with the majority of existing studies on cut roses, which utilise a single stem per vase.

Finally, to reduce potential subjectivity in visual observation, visual logs are recorded throughout the experiment and subsequently analysed using ImageJ, which is an open-source image analysis software commonly used in botanical research to quantify colour changes and morphological features. This procedure is expected to improve the objectivity of the evaluation process.

6.3 Increase of Replications

The number of replications was increased from 3 to 8 following a revised power analysis based on pilot study findings, as detailed in Chapter 4. This adjustment ensures that the experiment achieves the required level of statistical power.

6.4 Adjusted Timeline

As a result of restructuring the power analysis based on findings from prior studies, the overall experimental timeline has been delayed relative to the original plan. Although the timeline has been postponed, the revised structure should ultimately yield more precise and reliable results.

7. Ethics and Safety Concerns

The experiment involves only the two team members who observe and record rose conditions. There are no external participants, no consumption, and no physical risk. Therefore, no specific safety or ethics measures are necessary.

8. Project Timeline

29 May: Purchase roses. Begin experiment (Replications 1–8)

29 May – 7 June: Water replaced every two days. Experimental conditions and rose status recorded twice daily. Experiment terminated once all vases no longer meet the freshness standard

8 June – 10 June: Raw data organised and cross-checked against visual logs using ImageJ to verify recorded observations

11 June – 12 June: Exploratory analysis conducted; data cleaned, visualised, and checked for outliers

13 June – 16 June: Formal statistical analysis conducted based on collected data, with pilot study findings incorporated to support interpretation

17 June – 20 June: Final report written and submitted

20 June – 22 June: PowerPoint slides prepared and presentation rehearsed

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