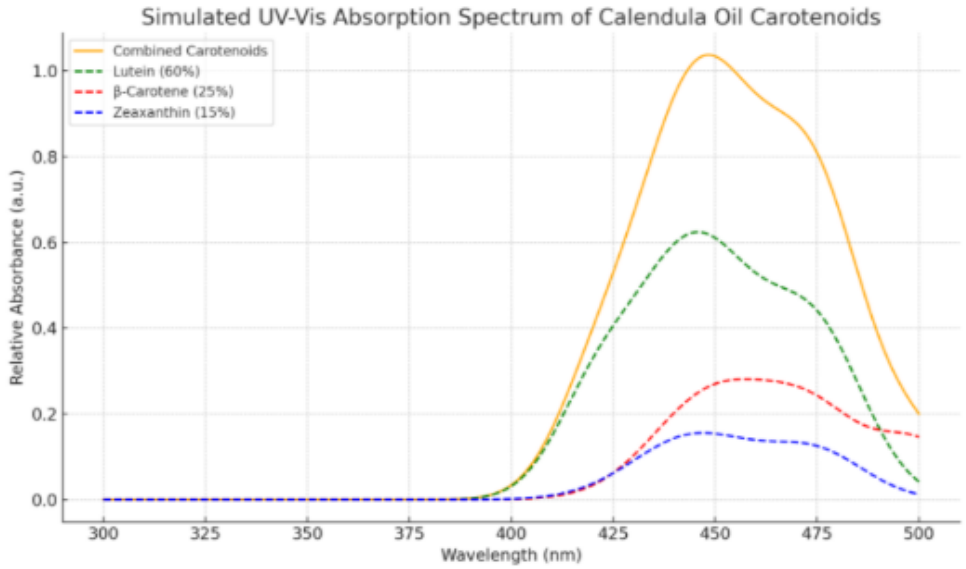
# Introduction

The objective of this experiment was to determine the optimal maceration time for extracting oil-soluble components from calendula (Calendula officinalis) petals into apricot kernel oil. Several infused oil samples, along with a blank control oil, were provided for analysis.

Each sample was diluted in an appropriate organic solvent, and optical density (OD) was measured at 450 nm — the absorption maximum of lutein, the major carotenoid in calendula oil extracts. In addition to lutein (~60% of total carotenoids), other carotenoids contributing to absorption in the 300–500 nm range include β-carotene (~25%) and zeaxanthin (~15%).

A simulated UV–Vis absorption spectrum of the principal carotenoids in calendula-infused oil is shown below:




In *Calendula officinalis*, approximately 70% of lutein occurs as esters with long-chain fatty acids. The UV–Vis molar absorptivity at 450 nm of esterified lutein differs only slightly from that of free lutein; therefore, the saponification step was omitted without compromising the accuracy of the study. Carotenoid concentrations were determined from optical density measurements at 450 nm using a calibration curve prepared with lutein-spiked oil standards. Results are reported as total carotenoid content, expressed in lutein equivalents (non-saponified samples).

# Receipt and Storage of Test Samples

Samples were received on August 5, 2025, and placed in a refrigerator at a temperature between 4 and 8°C. The samples were transported at room temperature, protected from light, to the lab facility in Hamilton and stored in a refrigerator at a temperature between 4 and 8°C for the duration of the experiment. Light exposure was limited by dark glass bottles and handling away from direct sunlight.

# Description of Test Samples

- **Test 1**: Apricot kernel oil used to prepare samples Test 2, Test 3, and Test 4
- **Test 2**: Apricot kernel oil was infused for 4 weeks, filtered, and no additional flowers were added
- **Test 3**: Apricot kernel oil was infused for a total of 8 weeks, and no additional flowers were added
- **Test 4**: Apricot kernel oil from Test 3 was filtered, new calendula flowers were added, and the oil was infused for an additional 4 weeks.
- **Test 5**: Apricot kernel oil extract prepared earlier from a different lot of oil.

The same lot of calendula flowers was used for Tests 2 through 5.

# Experimental Section

Based on preliminary experiments, we chose to dilute 1.00 g of the Test samples with 7.00 mL of tetrahydrofuran (THF) solvent to obtain optical density (OD) at 450 nm readings within the linearity range of our UV spectrophotometer.

## Calibration curve

The calibration curve was prepared by spiking apricot kernel oil (Test 1) with a known amount of lutein standard. Lutein (1.6 mg) was solubilized in THF (total 25 mL) in a volumetric flask to make a Lutein Stock Solution. The calibration curve was constructed by mixing 1 g of Test 1 with an accurately measured volume of the Lutein Stock Solution and THF, as shown in Table 1. Blank solution was prepared by mixing 1 g of Test 1 with 7 mL of THF. The UV-Vis spectrum was registered for each standard dilution in the range from 300 nm to 500 nm against the Blank. OD at 450 nm was used to construct the standard curve.

| Volume of Lutein Stock Solution (mL) | Volume of THF (mL) | Lutein Concentration (%) | OD at 450 nm |
|---|---|---|---|
| 3.00 | 4.00 | 0.00309 | 1.77 |
| 2.25 | 4.75 | 0.00231 | 1.34 |
| 1.50 | 5.50 | 0.00154 | 0.905 |
| 0.75 | 6.25 | 0.000771 | 0.459 |

*Table 1: Preparation of Lutein Standard Curve*

### Measurement of Total Carotenoids Expressed as Non-Saponified Lutein Equivalents

Three independent measurements were performed on August 7, 13, and 19, 2025, to assess measurement precision and detect any drift caused by changes in sample composition during storage.

The Test 2-5 samples (1 g) were diluted with 7 mL THF and UV-VIS spectrum was registered in the range from 300 nm to 500 nm against Blank (as for the Standard Curve). Optical Density at 450 nm was used to calculate the concentration of total carotenoids, expressed as lutein equivalents (non-saponified). Three readings for the same Test sample were averaged, and the standard deviation was calculated to assess method precision.

## RESULTS AND Conclusions

### Lutein Standard Curve

The standard curve shown in Figure 1 shows a linear correlation and excellent precision of the OD (450 nm) readings.

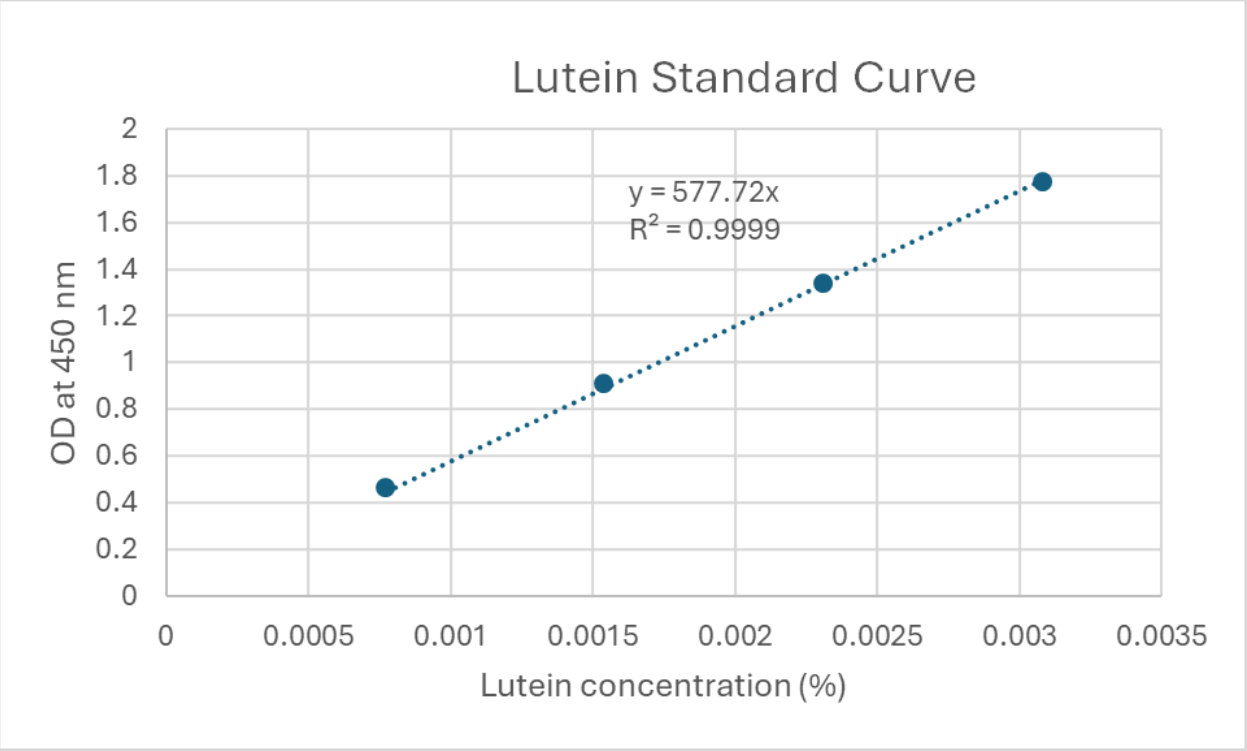



*Figure 1: Lutein Standard Curve*

## Measurement of Total Carotenoids in Test Samples

All the optical density (OD) readings were within the range of the standard curve. The measurements were repeated three times, six days apart, and the differences between them were very small. This shows that the method is precise, and the samples remain stable during storage. The results are shown in Table 2 and Figure 2.

| Test Sample No. | Total Carotenoids (%) | | | | |
|---|---|---|---|---|---|
| | Measurement No. 1 | Measurement No. 2 | Measurement No. 3 | Average | Standard Deviation |
| 2 | 0.00278 | 0.00245 | 0.00255 | 0.00259 | 0.000170 |
| 3 | 0.00712 | 0.00636 | 0.00638 | 0.00662 | 0.000432 |
| 4 | 0.0161 | 0.0157 | 0.0158 | 0.0159 | 0.000193 |
| 5 | 0.0154 | 0.0163 | 0.0165 | 0.0161 | 0.000566 |

*Table 2: Determination of Total Carotenoids Expressed as Lutein Equivalents (Non-Saponified) in Test Samples 2-5*

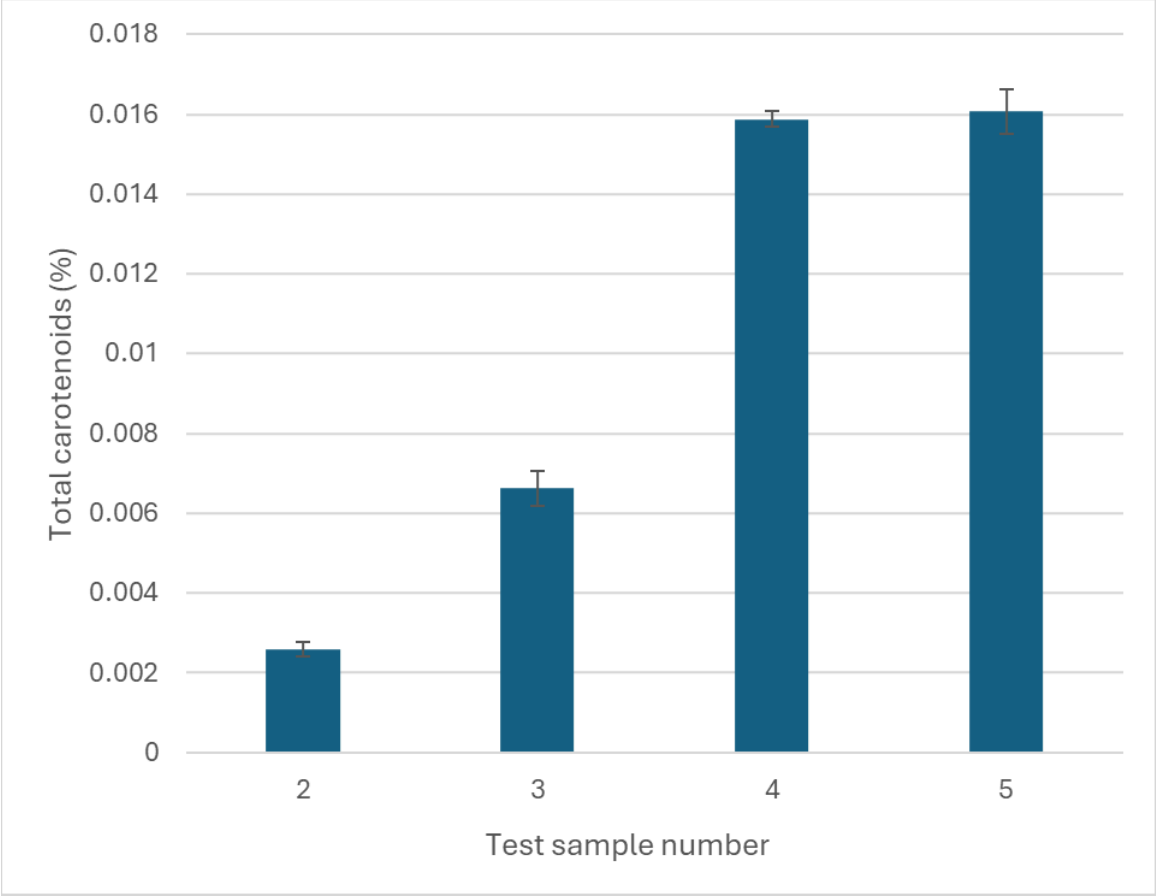



*Figure 2: Total Carotenoids Expressed as Lutein Equivalents (Non-Saponified) - average of three tests*

Infusing calendula flowers into apricot kernel oil successfully extracted carotenoids and probably other oil-soluble compounds from the flowers. The UV-Vis spectrum of Test 4 (Fig. 3) matches what we expect for calendula-infused oils.

For extractions using apricot kernel oil, the total carotenoid levels went up slightly more than linearly between 4 and 8 weeks. This means the oil hadn't yet reached its maximum capacity for carotenoids, so letting the infusion run longer than 8 weeks would likely extract even more.

When old plant material was replaced with fresh calendula after 8 weeks and the infusion continued for 4 more weeks, the carotenoid levels further increased by about 70% more than expected. Since it was the same batch of plant material, this shows that extraction depends not just on time but also on how quickly the plant material breaks down and possibly other factors like plant water content, mixing speed, temperature, and the ratio of oil to plant material.

Test 5, which had been stored at room temperature for around 5 months, had unexpectedly high carotenoid levels for a 4-week infusion. Some chemical changes may have occurred during storage. Comparing the UV-Vis scans of Test 4 and Test 5 shows a shift to higher wavelengths, but without a scan from when Test 5 was freshly made, it's hard to draw conclusions. Tests 2, 3, and 4 show very similar absorption profiles.

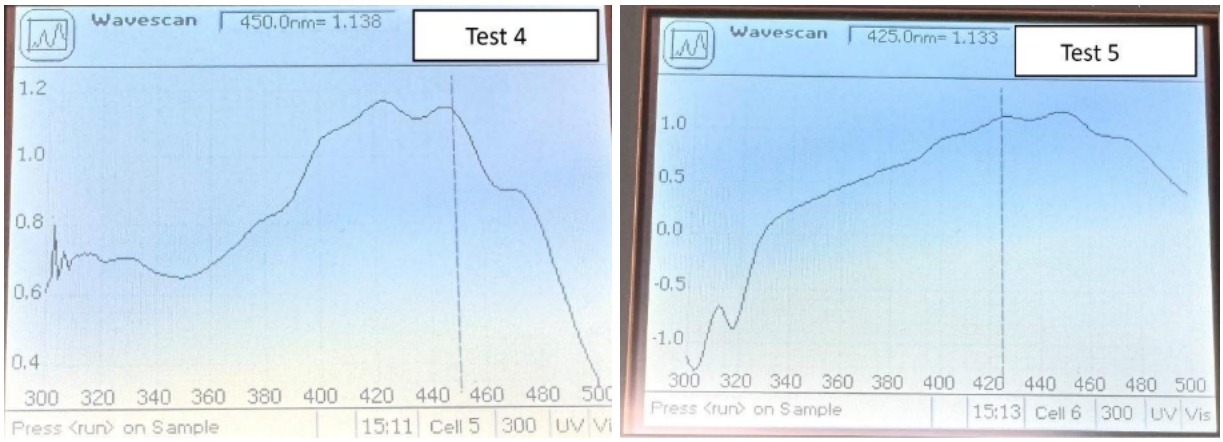



*Figure 3: UV-Vis scan of sample Test 4 compared to Test 5.*

We conducted an additional experiment to assess whether the infused oils remain stable at room temperature for one month. Portions of samples 1–4 were removed from the fridge and stored at room temperature for one month. After this period, measurements were repeated on both the refrigerated samples and the room-temperature samples, following the same procedure described above.

Figure 4 shows the results. The first (dotted) bars represent the original measurements, the blue bars show the measurements after additional storage in the fridge, and the red bars represent

the samples stored at room temperature. As evident from the graph, the results for each sample—regardless of storage conditions—differ only slightly, and the variation falls within the measurement error. The UV scans were also nearly identical.

We therefore conclude that short-term storage (up to one month) at room temperature, provided light is excluded, does not significantly affect the total carotenoid content.

We recommend testing whether stirring the oil during infusion could extract more carotenoids and make the process faster.

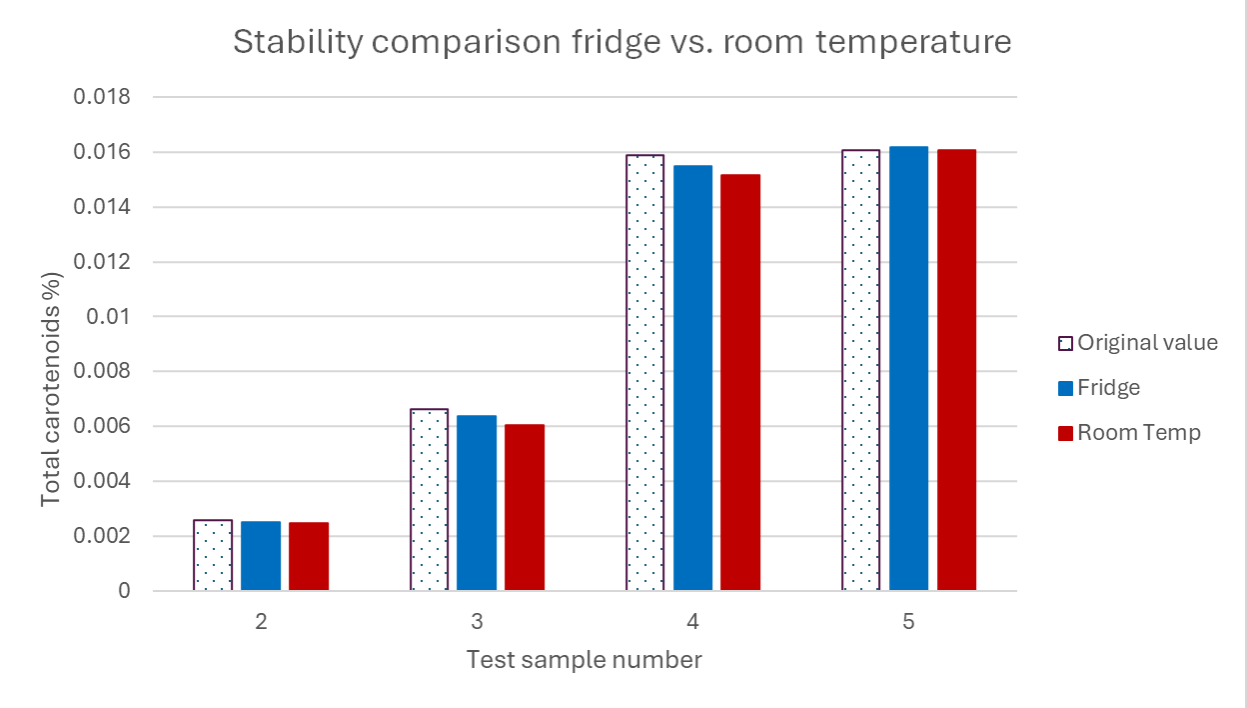



*Figure 4; Stability comparison: room temperature and fridge storage for one month*