Determination of Total Carotenoids in Calendula-Infused Oil by UV-Vis Spectrophotometry: The Effect of Continuous Mechanical Mixing During Infusion

Introduction

The objective of this experiment was to determine whether continuous mechanical shaking results in more efficient extraction of carotenoids from dried calendula flowers compared to once-daily manual mixing.

A method described in the previous report was used.

Receipt and Storage of Test Samples

Samples were received on December 19, 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 was infused for 1 month (Oct 17-Nov 17) with manual mixing every day

Test 2: Apricot kernel oil was infused for 1 month (Oct 17-Nov 17) with continuous mechanical shaking at 112 rpm

Test 3: Apricot kernel oil was infused for 1 month (Oct 17-Dec 17) with manual mixing every day

Test 4: Apricot kernel oil was infused for 1 month (Oct 17-DEc 17) with continuous mechanical shaking at 112 rpm

The same batch of calendula flowers was used for all experiments.

Experimental Section

The measurement method described in the previous report was used. Two independent measurements of each sample were performed on January 5rd, 2026.

Results and Conclusions

Infusion of calendula flowers into apricot kernel oil successfully extracted carotenoids from the plant material in all cases. Contrary to expectations, continuous mechanical shaking resulted in lower carotenoid levels than once-daily manual mixing for both the one-month and two-month extraction periods. Extending the infusion time from one to two months increased carotenoid extraction by approximately 32% for samples mixed manually once per day and by approximately 26% for samples subjected to continuous mechanical shaking. These results suggest that extending the infusion beyond two months would be unlikely to yield a meaningful increase in carotenoid recovery.

Although it might seem that constant shaking would extract more material, carotenoids are delicate compounds and can be easily damaged. Continuous shaking likely introduced more air

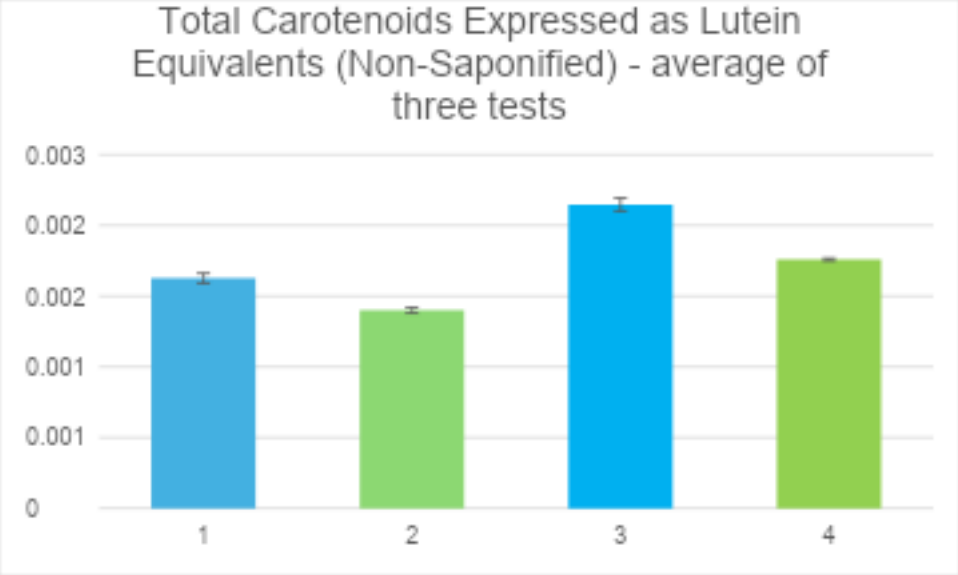
into the oil over time, which can slowly break down carotenoids and reduce the final amount measured.

It is also possible that continuous mechanical shaking stressed the plant material, breaking it into smaller particles, which may have caused some of the carotenoids to remain trapped within the plant matter.

Overall, the results suggest that slower, gentler extraction methods are better suited for extracting sensitive natural compounds like carotenoids into oil.

Table 1: Determination of Total Carotenoids Expressed as Lutein Equivalents (Non-Saponified) in Test Samples 2-5

Test Sample No.	Total Carotenoids (%)				
	Measurement No. 1	**Measurement No. 2**	**Average**		**Standard Deviation**
1	0.001601712	0.001651596	**0.00163**		3.53 E-05
2	0.001413716	0.001386283	**0.00140**		1.94 E-05
3	0.002180245	0.002114725	**0.00215**		4.63 E-05
4	0.001764009	0.001752825	**0.00176**		7.91 E-06



This work was performed in Stoic Beauty Inc. laboratory, 376 Mill Street Unit 3, Dundas, Ontario, Canada L9H 2M1

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Date: January 28, 2026

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