

# Optimization of stability and coloring power of double emulsion systems loaded with *Daucus carota* L.

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## Motivation and Objectives

**Anthocyanins (ACNs)** are water-soluble bioactive flavonoids present in flowers, fruits, and vegetables. **ACNs cover a wide palette of colors**, from blue and purple through orange to red. They are used in the **food industry (food colorant with E163 code)** in fruit juice concentrates, jellies, yogurts, marmalades, potato chips, ice creams, and soft drinks, **replacing the synthetic Red 40 colorants**. They also have diversified pharmaceutical and cosmetic uses due to their coloring and antioxidant attributes. However, their **instability due to pH, light, and temperature is a major concern**. This study used a **double water-in-oil-in-water (W<sub>1</sub>/O/W<sub>2</sub>) emulsion technique** to prevent rapid color loss from a commercial black carrot extract. A **Central Composite Rotatable Design (CCRD) 2<sup>2</sup>** examined the effects of colorant concentration (wt.%) and emulsion ratio on stability parameters (droplet size (D<sub>4,3</sub>), creaming index (CI), and colorimetric values (L\*, a\*). **The main objective was to find an optimized formulation regarding colorant potential and emulsion stability**. Measurements were taken at 1, 7, 15, and 30 days. **The optimal experimental design revealed two optimal solutions: 1) 48/52 (W<sub>1</sub>/O)/W<sub>2</sub> ratio with 6 wt.% colorant, and 2) 41/59 (W<sub>1</sub>/O)/W<sub>2</sub> ratio with 11 wt.% colorant**. These formulations showed lower creaming index and droplet sizes with higher colorant potential. After model validation, **the optimal condition was 6 wt.% colorant concentration and 48/52 v/v double emulsion ratio**. This maintained **stable color (L\*: 44.11±0.03, a\*: 25.79±0.01, D<sub>4,3</sub>: 9.62 ± 0.1 μm, and CI: 14.55±0.99%) after 30 days**. These findings are promising and might apply to several industries, such as food matrices and functional cosmetic agents.

## Methodology

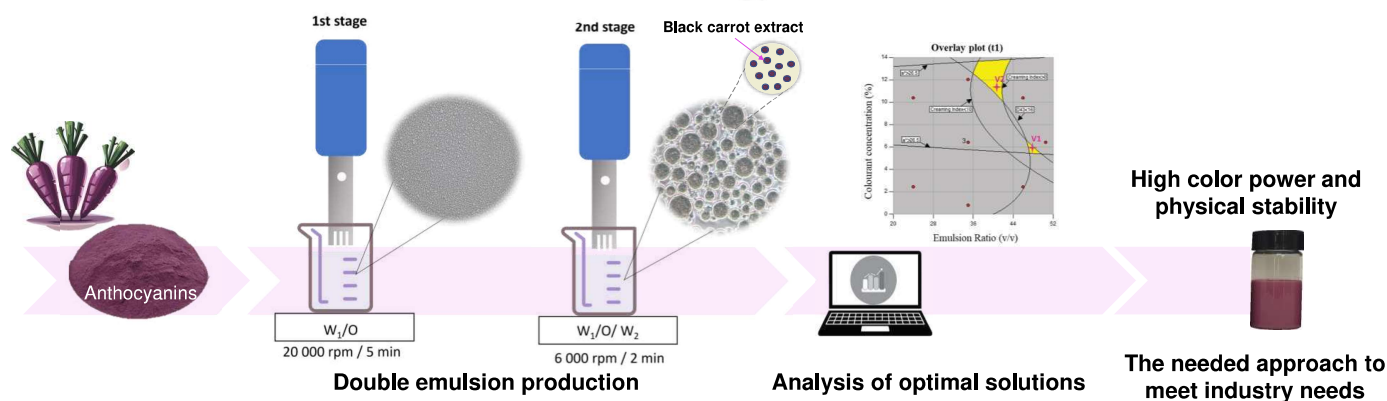


Fig. 1 Summary of the methodologies used in the study.

## Results

**Table 1.** Central Composite Rotatable Design (CCRD) 2<sup>2</sup> matrix with the coded levels and real values (in parentheses). X<sub>1</sub>: emulsion ratio ((W<sub>1</sub>/O)/W<sub>2</sub>) (v/v); X<sub>2</sub>: colorant concentration (wt.%).

Run	Independent Variables	
	Emulsion ratio (W <sub>1</sub> /O)/W <sub>2</sub> (v/v)	Colorant concentration (wt.%)
	X <sub>1</sub> (X <sub>1</sub> )	X <sub>2</sub> (X <sub>2</sub> )
E1	-1 (24/76)	-1 (2.43)
E2	+1 (46/54)	-1 (2.43)
E3	-1 (24/76)	+1 (10.37)
E4	+1 (46/54)	+1 (10.37)
E5	-1.41 (20/80)	0 (6.40)
E6	+1.41 (50/50)	0 (6.40)
E7	0 (35/65)	-1.41 (0.80)
E8	0 (35/65)	+1.41 (12.00)
E9	0 (35/65)	0 (6.40)
E10	0 (35/65)	0 (6.40)
E11	0 (35/65)	0 (6.40)

### Volume-mean droplet size

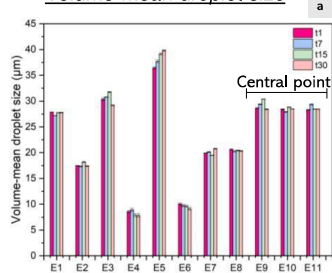
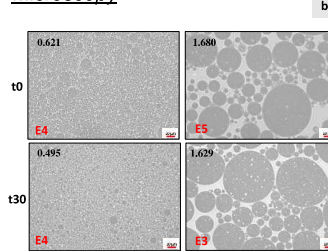


Fig. 3 (a) Results of the 11 experimental runs (emulsions) obtained by the Central Composite Rotatable Design 22 for D<sub>4,3</sub> over time: 1 day (t1); 7 days (t7); 15 days (t15) and 30 days (t30). (b) Microscopy images comparing the samples with smaller (E4) and larger (E5 and E3) droplet sizes at time 0 and after 30 days. The span values, indicating sample size homogeneity, are in bold in the upper left corner.

### Microscopy



D<sub>4,3</sub> changed minimally over time for all runs. E5 (20/80/6.40) had a greater Span value than E4 (46/54/10.37) (0.621). Sample E4 had the lowest value (0.495) for 30 days, while E3 had the highest value (1.629). **Optic microscopy (Fig 3b)** shows that each large droplet contains tiny droplets, creating a "droplet in droplet" structure, a typical double emulsion morphology.

### Analysis of optimal solution

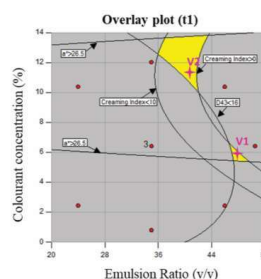


Fig 4. Overlay of the contour plots for D<sub>4,3</sub>, CI, a\*, and L\* depicting the optimum conditions (yellow area). The droplet size was set smaller than 16 μm, 0-creaming index <10%, a\* >26.5, and L\* within the experimental range.

Graphically selected solution for lower yellow ideal region: (W<sub>1</sub>/O)/W<sub>2</sub> ratio of 48/52 and colorant concentration of 6 wt.% (V1). For the upper yellow optimum region, (W<sub>1</sub>/O)/W<sub>2</sub> ratio was 41/59 and colorant concentration was 11 wt.% (V2).

Comparing V1 with V2, the emulsion V1 had significantly greater values of D<sub>4,3</sub> and CI; and lower value of a\*, pointing out that **V1 is the emulsion with the best characteristics, namely the lowest droplet size and lower creaming index, independently of the storage time.**

### Emulsions characterization



Fig. 2 Results of the 11 experimental runs (emulsions) obtained by the Central Composite Rotatable Design 22 for Creaming Index (CI) over time: t1; t7; t15, and t30.

E4 (46/54/10.37) had the lowest CI throughout the research, thus being the most stable. After 30 days, E5 (20/80/6.40) and E7 (35/65/0.80) had higher CI values of 61% and 62%, respectively.

### Color aspects

E7 (35/65/0.80) had the **lowest a\*** values and **highest L\*** values (t1: a\* = 17.09±0.06, L\* = 62.01±0.04; t30: a\* = 14.17±0.05, L\* = 63.07±0.13), indicating lower colorant power, while E4 (46/54/10.37) had the **highest a\*** values and **lowest L\*** values (t1: a\* = 28.92±0.07, L\* = 41.44±0.05; t30: a\* = 28.98±0.001, indicating higher colorant power.

## Conclusions

The study prioritized the development of colorant systems with robust colorant power and physical stability, essential for reliable performance in product applications. Using a Central Composite Rotatable Design (CCRD) 2<sup>2</sup>, the study pointed two promising formulations. The most effective formulation featured a 48/52 (W<sub>1</sub>/O)/W<sub>2</sub> ratio with a 6 wt.% colorant concentration. The one showed excellent physical stability, maintaining consistent coloring without leakage into the outer aqueous phase. This formulation provides new options for natural colorants in consumer products, encouraging healthier alternatives without losing quality.

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