

Formation and stabilisation of oil-in-water nanoemulsions using binary emulsifier mixtures of *Tribulus terrestris* extract and Quillaja bark saponin

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INTRODUCTION

Consumers' appetite to buy more sustainable, natural, and environmentally friendly ingredients is increasing due to environmental and social awareness and healthy habits [1]. Saponins are a class of natural surface-active substances, where most studies address the Quillaja Saponaria Molina tree (QS) as source. Although it proved to be an excellent emulsifier, there is a current need to identify and apply alternative saponin sources mainly due to sustainability, cost, and prevention of plant overexploitation. The saponin-rich extract *Tribulus terrestris* (TT) can be highlighted as a promising alternative [2].

OBJECTIVE

This work aims to study the effect of combining TT saponin-rich extract with Quillaja bark saponin in forming (and stabilising) nanoemulsions applying an experimental design methodology. A nanoemulsion characterisation comprising particle size, zeta potential, morphology by optical microscopy, and storage stability (30 days) was carried out.

EXPERIMENTAL

A series of oil-in-water (O/W) nanoemulsions were prepared using a fixed O/W ratio (w/w) of 10/90. For the preparation, the oil and water phases were mixed using an Ultraturrax at 11000 rpm for 3 min. This coarse emulsion was after subjected to high-pressure homogenisation (HPH) at 100 MPa. The prepared nanoemulsions were fully characterised. A 2^k full factorial design was performed with three factors and three replicates at the central point. Three independent factors were evaluated, namely the TT content in the emulsifier mixture (X1, 50-90 % wt.), the emulsifier content in the emulsion (X2, 1.5-4.5 % wt.), and the number of HPH cycles (X3, 5-15 cycles), whose effects were studied on selected responses (droplet diameter, D[3,2], (Y1, nm), and stability (Y2, days)).

RESULTS

The collected data are shown in Table 1. Excepting TQ6, all nanoemulsions appeared milky-like without phase separation. The stability showed to be favoured when the emulsifier content increases and TT content decreases. The maximum CI value (10%) was observed for TQ6 and TQ10. Both presented large droplet diameters, which according to Stokes' law, caused stability to decrease.

Table 1. Zeta Potential, droplet diameter, stability, and CI for the 11 samples (TQ1-TQ11) of the experimental design.

	Zeta Potential (mV)	Droplet Diameter (nm)	Stability (days)	CI 30 days (%)
TQ1	-44.0	215	11	6
TQ2	-43.5	78	30	0
TQ3	-41.1	103	30	0
TQ4	-46.6	112	7	8
TQ5	-44.0	197	10	6
TQ6	-46.4	921	0	10
TQ7	-43.9	169	10	6
TQ8	-41.2	493	4	8
TQ9	-46.8	210	4	8
TQ10	-43.6	493	4	10
TQ11	-45.4	892	2	8

Three most representative samples (TQ3, TQ7, and TQ11) are shown in Figure 1. The sample TQ3 presented one of the minor droplet diameters and maintained even after 30 days. By contrast, TQ7 showed a small droplet diameter after production, but evidenced considerable growth over time. TQ11 resulted in phase separation with an upper phase rich in oil, characterised by large oil droplets.

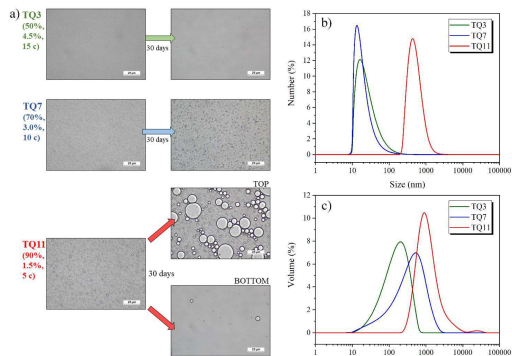


Figure 1. Morphological and size analysis of samples TQ3, TQ7, and TQ11: (a) optical microscopy (400x) at 0 and 30 days, (b) droplet size distribution in number, and (c) droplet size in volume.

The statistical analysis represented in Figure 2 and Equations (1) and (2) pointed out that for both dependent variables (i.e., D[3,2] and stability), the number of cycles did not have a significant effect (P-value > 0.05). So, these terms were not included in the final models. X1 and X2 parameters, significantly influence emulsions' mean droplet diameter, TT content being the most relevant variable. The size reduction was observed as the emulsifier increased and the TT content decreased. Stability was also selected to evaluate the emulsions' potential, and their variability within all the tested parameters was small. The highest stability occurs at higher emulsifier percentages (4.5%) and when the TT content reaches 50%.

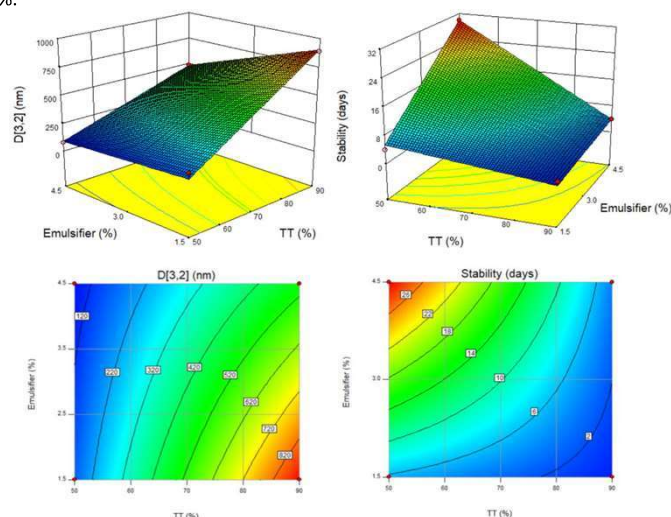


Figure 2. Response surfaces of the models established for the mean droplet diameter and the stability in the experimental region evaluated: 3D plots and contour plots for TT (%) and emulsifier (%) (minimum, central, and maximum levels).

$$Y_{D[3,2]} = 412.80 + 286.95 X_1 - 120.95 X_2 - 85.80 X_1 X_2 \quad (1)$$

$$Y_{Stability} = 10.13 - 7.62 X_1 + 6.88 X_2 - 5.38 X_1 X_2 \quad (2)$$

CONCLUSIONS

Nanoemulsions were successfully produced using emulsifier mixtures of TT extract with QS. The most stable samples (CI of 0% for 30 days) were achieved using an emulsifier mixture comprising TT at 50% wt. and a content of 4.5% wt. The samples were produced using 5 (TQ2) and 15 HPH (TQ3) cycles, achieving 78 and 103 nm droplet diameters, respectively. The performed design of experiments using as responses the droplet diameter and stability pointed out that the optimum samples need emulsifier contents between 3.9 and 4.5% wt., and emulsifier mixtures with 50 to 56% wt. of TT content.

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