ChemPor 2023

14th International Chemical and Biological Engineering Conference

Book of Abstracts

Instituto Politécnico de Bragança | September 12-15

ORDEM DOS ENGENHEIROS

ipb INSTITUTO POLITÉCNICO DE BRAGANÇA

This volume contains the extended abstracts presented at the 14th International Chemical and Biological Engineering Conference (CHEMPOR 2023), held in Bragança - Portugal, from the 12th to the 15th of September, 2023.

Instituto Politécnico de Bragança & Ordem dos Engenheiros

14th International Chemical and Biological Engineering Conference

(CHEMPOR-2023)

Book of Abstracts

Edited by:

Ana Maria Alves Queiroz da Silva

António Manuel Coelho Lino Peres

António Manuel Esteves Ribeiro

Maria Filomena Filipe Barreiro

Maria Olga de Amorim e Sá Ferreira

Paulo Miguel Pereira de Brito

Simão Pedro de Almeida Pinho

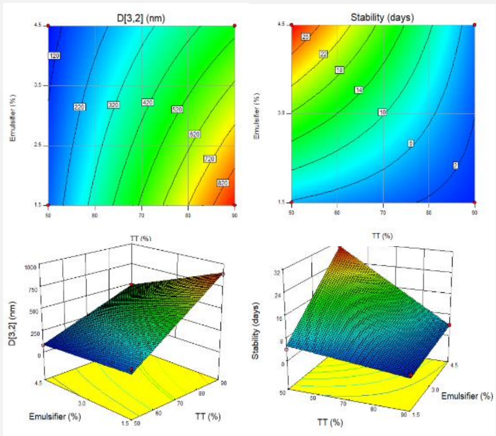
INSTITUTO POLITÉCNICO DE BRAGANÇA

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

*T.B. Schreiner[1,2,3,4], A. Santamaria-Echart[1,2], A.M. Peres[1,2], M.M. Dias[3,4], S.P. Pinho[1,2], M.F. Barreiro[1,2]**

[1]Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; [2]Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (LA SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal; [3]Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Materials (LSRE-LCM), Faculty of Engineering University of Porto, Rua Dr. Roberto Frias, S/N, 4200-465 Porto, Portugal; [4]Associate Laboratory in Chemical Engineering (ALiCE), Faculdade de Engenharia, Universidade do Porto, R. Dr. Roberto Frias, S/N, 4200-465, Porto, Portugal.

**barreiro@ipb.pt*



Emulsifiers are used in many industrial fields, with the natural origin ones gaining increasing relevance. Identifying diversified sources as natural emulsifiers is a pertinent topic for sustainability principles. Saponin-rich extract *Tribulus terrestris* (TT) was evaluated as a viable alternative to *Quillaja* bark saponin (QS). An experimental design using binary emulsifier mixtures of TT with QS was carried out by varying their composition, content, and processing conditions (HPH cycles). Emulsions were characterised by zeta potential, morphology, droplet size, and stability. Zeta potential indicated stable emulsions; even creaming was observed in samples with a low emulsifier and high TT%. Diameter of emulsions was between 78-921 nm, with smaller sizes agreeing with the higher stability. Statistical analysis revealed an optimum composition range of emulsifier (3.9-4.5%wt.) and TT content(50-56%wt.) to reach stable products. Overall, TT can provide an effective solution combined with QS.

Introduction

Nanoemulsions are colloidal dispersion systems comprising two immiscible phases and an emulsifier resulting in a single macroscopic phase. They have small-sized droplets and can be kinetically stable over long time scales. The emulsifier is the main ingredient responsible for maintaining the stability and performance of emulsified products [1]. However, most commercialised products use synthetic emulsifiers. Nevertheless, consumers' appetence to buy more sustainable, natural, and environmentally friendly ingredients is increasing due to environmental and social awareness and healthy habits [2]. In this context, saponins are a class of natural surface-active substances versatile in terms of physicochemical and biological properties. Most studies address saponins from the *Quillaja* Saponaria Molina tree (QS). 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) from puncture vine can be highlighted as a promising alternative, presenting a critical micelle concentration (CMC) of 0.59 g/L, circa 30% lower than other studied extracts, and superior emulsifying capacity/stability and foaming capacity/stability [3].

Objectives

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.

Methods

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 2k 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 (X_1, 50-90 % wt.), the emulsifier content in the emulsion (X_2, 1.5-4.5 % wt.), and the number of HPH cycles (X_3, 5-15 cycles), whose effects were studied on selected responses (droplet diameter, D[3,2], (Y_1, nm), and stability (Y_2, days)).

Results

The collected data are shown in Table 1. Excepting TQ6 all nanoemulsions appeared milky-like without phase separation. That specific samples showed a CI value of 2% right after preparation. This fact could be associated with the lower emulsifier content (1.5% wt.) and the higher TT content (90% wt.) of the used emulsifier mixture. The zeta potential ranged from -47 to -41 mV, indicative of sample stability. However, some prepared samples did not remain stable for a 30 days-period, even when presenting highly negative initial zeta potential values. Although this parameter is generally a suitable indicator of stability, other factors, such as macroscopic inspection, must be conducted to guarantee highly stable emulsions. In this work, such stability parameters were monitored over time. The samples corresponding to the central point (samples TQ1, TQ5, and TQ7) presented a slightly higher emulsifier content (3.0% wt.) and a lower TT content (70% wt.), revealing intermediate stability (10 days). This behaviour indicates that stability is favoured when the emulsifier content increases and TT content decreases (a CI of only 6% was determined after 30 days). 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

The morphology of the emulsions was analysed by optical microscopy. Generally, on the first day, most samples showed droplets of round shape and high homogeneity. The sample TQ3 presented one of the minor droplet diameters, making its visualisation difficult. This morphological aspect was maintained even after 30 days, in accordance with the observed high stability (CI of 0% after 30 days). By contrast, the sample TQ7, which showed a small droplet diameter after production, evidenced considerable growth over time. This effect is reflected in a CI of 6%. One of the most unstable samples, TQ11, which promptly resulted in two separated phases after production, has shown an upper phase rich in oil, characterised by large oil droplets (original dispersed phase) dispersed in water.

The experimental droplet size measurements enabled the analysis of the effect of the obtained formulations. The TQ2 and TQ3 samples showed the lowest values, 78 and 103 nm, respectively, favouring the stability of the emulsions against aggregation and creaming since the droplets' rate movement was proportional to the square of their radius, according to Stokes' law [2]. The triplicates of the central point presented similar values, with an average of 194 nm, reflecting the excellent reproducibility of the experiments. The less stable samples (TQ6, TQ8, TQ10, and TQ11) reached the largest diameters, where samples TQ6 and TQ11 have a size around 900 nm, again in agreement with Stokes' law. The statistical regression parameters are shown in Table 2. The analysis pointed out that for both dependent variables (i.e., D[3,2] and stability), the number of cycles did not have a significant effect, nor did none of the respective second and third-order parameters (P-value > 0.05). So, these terms were not included in the final models. X_1 and X_2 parameters, also shown in Table 2, 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%, as shown in the Graphical Abstract.

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.

Table 2. Regression parameters (β's coefficients) of the optimal Multiple Linear Regression Models were established for the droplet diameter (nm) and stability with time (days) using a stepwise variable selection method for the 2^3 experimental design and respective model quality parameters.

Source	Droplet diameter D[3,2](nm)		Stability (days)	
	β's coefficients (coded factors)	*P*-value	β's coefficients (coded factors)	*P*-value
Model	----	<0.0001	----	<0.0001
Intercept	+412.80	<0.0001	+10.13	<0.0001
X_1 – *Tribulus terrestris* (%)	+286.95	<0.0001	-7.62	<0.0001
X_2 – Emulsifier (%)	-120.95	<0.0001	+6.88	<0.0001
X_3 – Number of cycles	-	n.s.	-	n.s.
X_1X_2	-85.80	0.0003	-5.38	<0.0001
Curvature	-----	<0.0001	-----	0.7877
Lack of fit	----	0.2992	----	0.1774
Quality parameter	**Values**		**Values**	
Adequate Precision	36.5		39.4	
R^2	0.9922		0.9934	
R^2_{adj}	0.9882		0.9901	
R^2_{pred}	0.9709		0.9746	

Acknowledgements

The authors are grateful to FCT for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020), SusTEC (LA/P/0007/2021), LSRE-LCM (UIDB/50020/2020 and UIDP/00690/2020), and ALiCE (LA/P/0045/2020). FCT for the PhD research grant of T.B. Schreiner (2020.05564.BD). National funding by FCT, P.I., through the institutional scientific employment program contract of A. Santamaria-Echart.

References

[1] Y. Yang et al., Food Hydrocolloids, 30 (2013) 589-96.
[2] D.J. McClements et al., Advances in Colloid and Interface Science, 234 (2016) 3-26.
[3] T.B. Schreiner et al., Colloids and Surfaces A: Physicochemical and Engineering Aspects, 623 (2021) 126748.