Appendix

Article

Development of a carotenoid-rich microalgae colorant by microencapsulation

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High pressure liquid chromatography (HPLC)

To identify the presence of *Dunaliella salina* carotenoids, an HPLC analysis of the particles was performed. The typical HPLC carotenoid profile is shown in **Figure S1**. This result confirms β-carotene as the major carotenoid in the particles. 9-cis-β-carotene was the principal form of carotene identified (compound 5). Lutein, zeaxanthin, α-carotene, and all-trans beta carotene were also identified (compound 1, 2, 3 and 4, respectively), in residual amount.

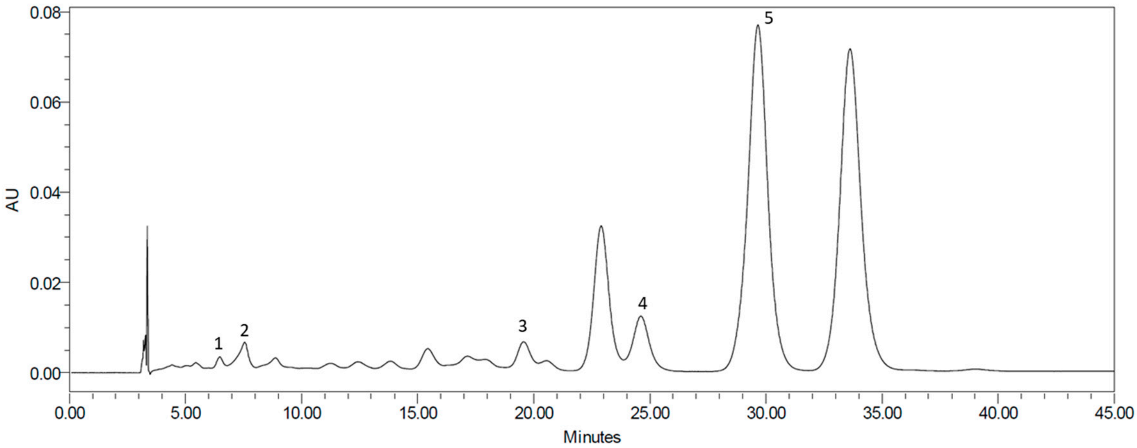

Figure S1. HPLC carotenoids profile at 450 nm of the particle produced with an emulsion oil concentration of 37%, 13500 rpm of emulsification stirring seed, and at 165°C of spray drying inlet air temperature. (1) lutein; (2) zeaxanthin; (3) α-carotene; (4) all-trans beta carotene and (5) 9-cis-β-carotene.

Experimental design and statistical analysis

A response surface methodology was used to evaluate the influence of process conditions on the encapsulation of ultra-high supercritical carbon dioxide ($scCO_2$) extract from *Dunaliella salina* integrating the o/w emulsification and spray drying. Several factors are known to affect some attributes of the intermediate products (fresh emulsion and dried particles) as well as some process-related aspects. These factors are illustrated in **Figure S2**.

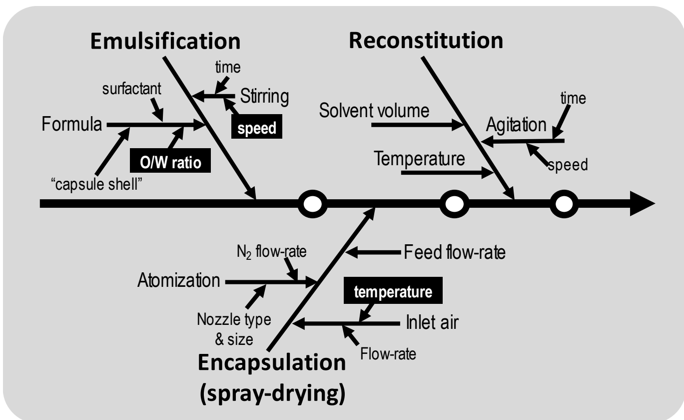

Figure S2. Ishikawa diagram displaying the variables impacting the reconstituted microcapsules dispersion attributes. The black-shaded variables were included in the multifactorial optimization.

Among all the potential factors, the effect of the emulsion oil concentration with respect to total solids (3–37 wt. %), the emulsification stirring speed (6500-21500 rpm), and the spray drying inlet air temperature (110–220°C) in several attributes of the fresh emulsion, the particles, and the reconstituted emulsion were studied (**Manuscript Table 1**).

The encapsulation yield and efficiency data resulting from the experiments performed according to the experimental conditions defined by the CCD were analyzed by using the Modde v.12 (Umetrics, Umeå, Sweden) software. The statistical tests, including the adjustments of the design model and factors effects, were considered to be significant when the resulting p-value was lower than the predefined $\alpha = 0.05$.

The underlying three-factor polynomial models include linear, two-factor interactions as well as quadratic terms as depicted by Eq. A1. In this equation, A, B, and C represent the independent variables, i.e., the emulsion oil concentration with respect to total solids, the emulsification stirring speed, and the spray drying inlet air temperature.

$$Y = a + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + c_1A^2 + c2B^2 + c3C^2 \quad (1)$$

The model coefficients (a, bx, and cx) were estimated by multivariate linear regression, and its significance assessed after performing the corresponding ANOVA (**Table S1**).

Table S1. Linear and interaction effects and respective significance levels (p) of the tested variables [factors: emulsion oil concentration with respect to total solids (A), emulsification stirring speed (B), and spray drying inlet air temperature (C)] and interactions on encapsulation yield and efficiency.

	Encapsulation yield		Encapsulation efficiency	
	Coeff. SC	*p* value	Coeff. SC	*p* value
Constant	42.4		41.4	
A	-12.7	1.76E-07	25.9	2.2E-08
B	2.7	0.052	-2.8	0.185
C	2.7	0.052	-2.5	0.239
AB			10.9	0.001

The response surfaces fitted to the encapsulation yield and efficiency (Manuscript Figure 1) can be described using a polynomial model as a function of emulsion oil concentration with respect to total solids (A), emulsification stirring speed (B) and spray drying inlet air temperature (C). In these response surfaces, the non-significant effects (**Table S1**) were removed from the complete model (**Eq. A1**) giving origin to the simplified models described in **Eq. A2** and **Eq. A3**.

$$\text{Encapsulation yield, wt.\%} = 42.4 – 12.7A + 2.7B + 2.7C \quad (2)$$
$$\text{Encapsulation efficiency, wt.\%} = 41.4 + 25.9A - 2.8B - 2.5C + 10.9AB \quad (3)$$

Table S2. ANOVA analysis for encapsulation yield and efficiency obtained using different spray drying emulsion conditions.

Encapsulation yield	DF	SS	MS	F	p-value	SD
Total	17	33119.0	1948.2			
Constant	1	30409.5	30409.5			
Total corrected	16	2709.5	169.3			13.0
Regression	3	2421.9	807.3	36.5	0.000	28.4
Residual	13	287.7	22.1			4.7
Lack of Fit (Model error)	11	287.0	26,1	78.3	0.013	5.1
Pure error (Replicate error)	2	0.7	0.3			0.6
	N = 17	Q2 =	0.798	Cond. no. =	1.1	
	DF = 13	R2 =	0.894	RSD =	4.7	
		R2 adj. =	0.869			
Encapsulation efficiency	DF	SS	MS	F	p-value	SD
Total	17	40179,4	2363.5			
Constant	1	29186,2	29186.2			
Total corrected	16	10993,2	687.1			26.2
Regression	4	10328,1	2582.0	46.6	0.000	50.8
Residual	12	665,094	55.4			7.4
Lack of Fit (Model error)	10	569,978	57.0	1.2	0.538	7.5
Pure error (Replicate error)	2	95,1152	47.5			6.9
	N = 17	Q2 =	0.869	Cond. no. =	1.5	
	DF = 12	R2 =	0.939	RSD =	7.4	
		R2 adj. =	0.919			

The values for R2 of these models suggest a good agreement between the experimental data and the values predicted by the model for the encapsulation yield and efficiency. About 87% and 92% of the observed overall variance concerning the encapsulation yield and efficiency respectively, are explained by these models (**Table S2**). The reproducibility of the model to the encapsulation yield and efficiency was 93% and 99% respectively, considering the center points.