Supporting Information

Single-step extraction of carotenoids from brown macroalgae using non-ionic surfactants

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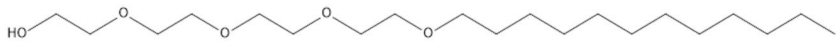
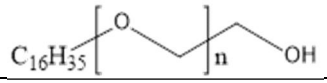
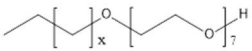
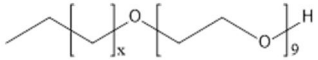
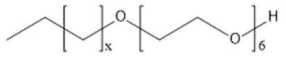
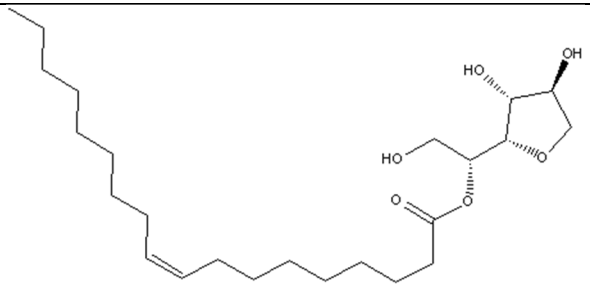
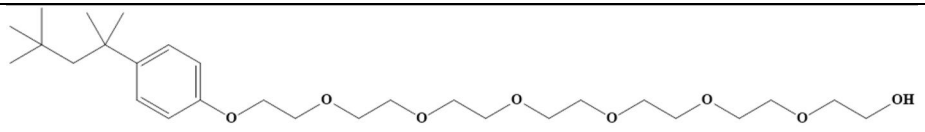
Table A1. Identification of each batch of biomass from *Sargassum muticum* wild harvested in Portugal and used in this work..

Batch reference	Harvest date	Water content (%)
S1.0315.D	13/01/2015	8.6
S1.4915.D	02/12/2015	6.2

Table A2. Table of acronyms, chemical formula and molecular structure of surfactants.

Surfactants Name	Chemical formula	Chemical Structure
Brij 30	$C_{20}H_{42}O_5$	
Brij 93	$C_{22}H_{44}O_3$	
Brij 98	$C_{20}H_{40}O_2$	
Tomadol 25-7 (x= 11-14)	C_{12}-C_{15} 7EO´S	
Tomadol 9-1 (x=10-12)	C_{10}-C_{12} 7EO´S	
Tomadol 91-6 (x=8-10)	C_8-C_{10} 7EO´S	
Tween 80	$C_{64}H_{124}O_{26}$	
Triton X-114	$C_{14}H_{22}O_X$ (x= 7 or 8)	

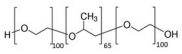
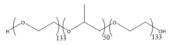
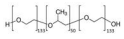
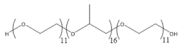
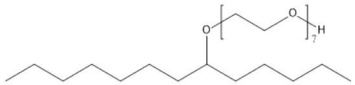
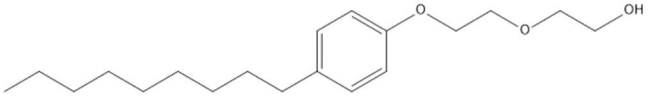
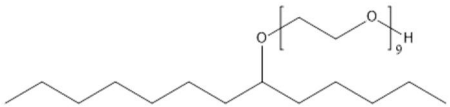
Pluronic F-127	HO $(CH_2CH_2O)_{100}$ $(CH_2CH(CH3)O)_{66}$ $(CH_2CH_2O)_{100}H$	
Pluronic F-108	HO $(CH_2CH_2O)_{133}$ $(CH_2CH(CH3)O)_{50}$ $(CH_2CH_2O)_{133}H$	
Pluronic P-123	HO $(CH_2CH_2O)_{20}$ $(CH_2CH(CH3)O)_{70}$ $(CH_2CH_2O)_{20}H$	
Pluronic L-35	$HO(CH_2CH2O)_{11}$-$CHCH_3CH_2O)_{16}$	
Tergitol 15-S-7	$C_{12-14}H_{25-29}O$ $[CH_2CH_2O]_7H$	
Tergitol NP-10	$C_{19}H_{32}O_3$	
Tergitol 15-S-9	$CH_3(CH_2)_{12–14}(OC_2H_4)_9OH$	

Table A3. Uncoded coefficients used in the 2^3 factorial planning experiments for Pluronic P-123 and Tomadol 25-7.

Variables	Axial Point	Factorial Point	Central Point	Factorial Point	Axial Point
	-1.68	**-1**	**0**	**1**	**1.68**
C_{surf} (mol/L)	0.2	0.5	1.0	1.5	1.8
t (minutes)	48	65	90	115	132
$R_{(S/L)}$	0.01	0.02	0.04	0.06	0.07

Table A4. Coded coefficients adopted in the 2^3 factorial planning for Tomadol 25-7 and Pluronic P-123.

Experiment	X_1	X_2	X_3
1	-1	-1	-1
2	1	-1	-1
3	-1	1	-1
4	1	1	-1
5	-1	-1	1
6	1	-1	1
7	-1	1	1
8	1	1	1
9	-1.68	0	0
10	1.68	0	0
11	0	-1.68	0
12	0	1.68	0
13	0	0	-1.68
14	0	0	1.68
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0

Table A5. 2^3 factorial planning for Pluronic P-123 using as independent variables C_{surf} (mol/L), *t* and $R_{(S/L)}$.

Experiment	C_{surf} (mol/L)	t (minutes)	$R_{(S/L)}$	$[Carotenoids]_{experimental}$ ($mg_{carotenoids}/g_{dried\ mass}$)	$[Carotenoids]_{predicted}$ ($mg_{carotenoids}/g_{dried\ mass}$)	Relative deviation (%)
1	0.005	65	0.02	0.42	0.39	7.14
2	0.015	65	0.02	0.49	0.46	6.12
3	0.005	115	0.02	0.33	0.33	0.00
4	0.015	115	0.02	0.40	0.42	-5.00
5	0.005	65	0.06	0.70	0.67	4.29
6	0.015	65	0.06	0.93	0.92	1.08
7	0.005	115	0.06	0.72	0.75	-4.17
8	0.015	115	0.06	0.99	1.02	-3.03
9	0.0016	90	0.04	0.42	0.44	-4.76
10	0.0184	90	0.04	0.73	0.72	1.37
11	0.01	48	0.04	0.57	0.63	-10.53
12	0.01	132	0.04	0.73	0.67	8.22
13	0.01	90	0.01	0.24	0.27	-12.50
14	0.01	90	0.07	1.03	1.01	1.94
15	0.01	90	0.04	0.70	0.65	7.14
16	0.01	90	0.04	0.68	0.65	4.41
17	0.01	90	0.04	0.69	0.65	5.80
18	0.01	90	0.04	0.62	0.65	-4.84
19	0.01	90	0.04	0.64	0.65	-1.56
20	0.01	90	0.04	0.55	0.65	-18.18

Table A6. Regression coefficients and standard deviation of the 2^3 factorial planning developed for the surfactant Pluronic P-123.

	Coefficients	***Standard* error**	***t(10)***	***p-value***
Intercept	1.17	0.10	11.73	0.00
C_{surf}	0.01	0.13	0.09	0.93
C_{surf}^2	-0.22	0.13	-1.71	0.12
t	0.12	0.13	0.87	0.40
t^2	-0.26	0.13	-1.98	0.08
$R_{(S/L)}$	-0.48	0.14	-3.50	0.01
$R_{(S/L)}^2$	0.22	0.15	1.44	0.18
$C_{surf} \times t$	-0.06	0.17	-0.35	0.74
$C_{surf} \times t$	0.03	0.17	0.18	0.86
$t \times R_{(S/L)}$	-0.09	0.17	-0.55	0.60

Table A7. ANOVA results of the 2^3 factorial planning developed for the surfactant Pluronic P-123.

	Sum Squares	**Degrees of Freedom**	**Mean Square**	**F_{cal}**	***p*-value**
Regression	1.34	9.00	0.15	2.49	0.09
Error	0.60	10.00	0.06		
Total	1.94				

Table A8. Results obtained for the percent recoveries found for the experimental points studied in the 2^3 factorial planning applied to Pluronic P-123, using the amount of carotenoids experimentally obtained and predicted by the model.

Experiment	C_{surf} (mol/L)	t (minutes)	$R_{(S/L)}$	$[Carotenoids]_{experimental}$ ($mg_{carotenoids}/g_{dried\ mass}$)	$[Carotenoids]_{predicted}$ ($mg_{carotenoids}/g_{dried\ mass}$)	Recovery (%) $[Carotenoids]_{experimental}$ / $[Carotenoids]_{predicted}$
1	0.0	65	0.02	0.42	1.15	37
2	0.015	65	0.02	0.49	1.19	41
3	0.005	115	0.02	0.33	1.42	23
4	0.015	115	0.02	0.40	1.35	30
5	0.005	65	0.06	0.70	0.73	96
6	0.015	65	0.06	0.93	0.84	111
7	0.005	115	0.06	0.72	0.81	88
8	0.015	115	0.06	0.99	0.80	124
9	0.0016	90	0.04	0.42	0.84	50
10	0.0184	90	0.04	0.73	0.86	85
11	0.01	48	0.04	0.57	0.71	81
12	0.01	132	0.04	0.73	0.90	80
13	0.01	90	0.01	0.24	1.78	14
14	0.01	90	0.07	1.03	1.05	98
15	0.01	90	0.04	0.70	1.16	60
16	0.01	90	0.04	0.68	1.16	59
17	0.01	90	0.04	0.69	1.16	59
18	0.01	90	0.04	0.62	1.16	53
19	0.01	90	0.04	0.64	1.16	55
20	0.01	90	0.04	0.55	1.16	48

Table A9. Data attributed to the independent variables [C_{surf}, *t*, and $R_{(S/L)}$] to define the 2^3 factorial planning for Tomadol 25-7 and respective results of concentration of carotenoids experimentally extracted, the predicted results found through the mathematical model developed and the respective relative deviation.

Experiment	C_{surf} (mol/L)	t (minutes)	$R_{(S/L)}$	$[Carotenoids]_{experimental}$ ($mg_{carotenoids}/g_{dried\ mass}$)	$[Carotenoids]_{predicted}$ ($mg_{carotenoids}/g_{dried\ mass}$)	Relative deviation (%)
1	0.005	65	0.02	0.42	0.39	8.19
2	0.015	65	0.02	0.49	0.46	7.23
3	0.005	115	0.02	0.33	0.33	-0.63
4	0.015	115	0.02	0.40	0.42	-5.89
5	0.005	65	0.06	0.70	0.67	3.95
6	0.015	65	0.06	0.93	0.92	0.66
7	0.005	115	0.06	0.72	0.75	-4.39
8	0.015	115	0.06	0.99	1.02	-3.09
9	0.0016	90	0.04	0.42	0.44	-3.58
10	0.0184	90	0.04	0.73	0.72	1.28
11	0.01	48	0.04	0.57	0.63	-10.48
12	0.01	132	0.04	0.73	0.67	7.48
13	0.01	90	0.01	0.24	0.27	-10.17
14	0.01	90	0.07	1.03	1.01	1.84
15	0.01	90	0.04	0.70	0.65	7.86
16	0.01	90	0.04	0.68	0.65	5.12
17	0.01	90	0.04	0.69	0.65	6.17

18	0.01	90	0.04	0.62	0.65	-4.89
19	0.01	90	0.04	0.64	0.65	-0.98
20	0.01	90	0.04	0.55	0.65	-17.27

Table A10. Regression coefficients of the predicted second-order polynomial model applied for the carotenoids extraction obtained from the RSM design using Tomadol 25-7 aqueous solutions.

	Coefficients	***Standard* error**	***t(10)***	***p-value***
intercept	0.65	0.02	29.14	0.00
C_{surf}	0.17	0.03	5.73	0.00
C_{surf}^2	-0.05	0.03	-1.72	0.12
t	0.02	0.03	0.78	0.45
t^2	0.00	0.03	0.09	0.93
$R_{(S/L)}$	0.44	0.03	14.89	0.00
$R_{(S/L)}^2$	-0.01	0.03	-0.30	0.77
$C_{surf} \times t$	0.01	0.04	0.30	0.77
$C_{surf} \times R_{(S/L)}$	0.09	0.04	2.31	0.04
$t \times R_{(S/L)}$	0.07	0.04	1.74	0.11

Table A11. ANOVA data for the extraction of carotenoids obtained from the factorial planning carried with Tomadol 25-7.

	Sum Squares	**Degrees of Freedom**	**Mean Square**	**F_{cal}**	***p* -value**
Regression	0.79	9.00	0.09	23.67	0.000014
Error	0.04	10.00	0.00		
Total	0.83				

Table A12. Data attributed to the independent variables [C_{surf} and $R_{(S/L)}$] to define the 2^2 factorial planning for Tomadol 25-7 and respective results of concentration of carotenoids experimentally extracted, the theoretical results found for the mathematical model developed and the respective relative deviation.

Experiment	C_{surf} (mol/L)	$R_{(S/L)}$	$[Carotenoids]_{experimental}$ ($mg_{carotenoids}/g_{dried\ mass}$)	$[Carotenoids]_{predicted}$ ($mg_{carotenoids}/g_{dried\ mass}$)	Relative deviation (%)
1	0.005	0.02	0.42	0.36	14.90
2	0.015	0.02	0.49	0.44	10.64
3	0.005	0.02	0.33	0.36	-8.93
4	0.015	0.02	0.40	0.44	-9.84
5	0.005	0.06	0.70	0.71	-1.62
6	0.015	0.06	0.93	0.97	-4.80
7	0.005	0.06	0.72	0.71	1.16
8	0.015	0.06	0.99	0.97	2.11
9	0.0016	0.04	0.42	0.44	-3.74
10	0.0184	0.04	0.73	0.72	1.19
11	0.01	0.04	0.57	0.65	-13.43
12	0.01	0.04	0.73	0.65	10.51
13	0.01	0.01	0.24	0.27	-10.45
14	0.01	0.07	1.03	1.01	1.78
15	0.01	0.04	0.70	0.65	7.71

16	0.01	0.04	0.68	0.65	4.97
17	0.01	0.04	0.69	0.65	6.02
18	0.01	0.04	0.62	0.65	-5.06
19	0.01	0.04	0.64	0.65	-1.14
20	0.01	0.04	0.55	0.65	-17.46

Table A13. ANOVA data for the extraction of carotenoids obtained from the new factorial planning with two independent variables, C_{surf} and $R_{(S/L)}$, carried with Tomadol 25-7.

	Sum of Squares	**Degrees of freedom**	**Mean**	**Fcal**	***p-value***
Regression	0.76	2.00	0.38	97.87	0.00
Error	0.07	17.00	0.00		
Total	0.83				

Table A14. Results obtained for the percent recoveries found for the experimental points studied in the 2^2 factorial planning applied to Tomadol 25-7, using the amount of carotenoids experimentally obtained and predicted by the model.

Experiment	C_{surf} (mol/L)	R (S/L)	$[Carotenoids]_{experimental}$ ($mg_{carotenoids}/g_{dried\ mass}$)	$[Carotenoids]_{predicted}$ ($mg_{carotenoids}/g_{dried\ mass}$)	Recovery (%) $[Carotenoids]_{experimental}$ / $[Carotenoids]_{predicted}$
1	0.005	0.02	0.42	0.36	118
2	0.015	0.02	0.49	0.44	112
3	0.005	0.02	0.33	0.36	92
4	0.015	0.02	0.40	0.44	91
5	0.005	0.06	0.70	0.71	98
6	0.015	0.06	0.93	0.97	95
7	0.005	0.06	0.72	0.71	101
8	0.015	0.06	0.99	0.97	102
9	0.0016	0.04	0.42	0.44	96
10	0.0184	0.04	0.73	0.72	101
11	0.01	0.04	0.57	0.65	88
12	0.01	0.04	0.73	0.65	112
13	0.01	0.01	0.24	0.27	91
14	0.01	0.07	1.03	1.01	102
15	0.01	0.04	0.70	0.65	108
16	0.01	0.04	0.68	0.65	105

17	0.01	0.04	0.69	0.65	106
18	0.01	0.04	0.62	0.65	95
19	0.01	0.04	0.64	0.65	99
20	0.01	0.04	0.55	0.65	85

Equation A1. Polynomial equation obtained for the 2^3 factorial planning carried for Pluronic P-123, with x, y and z representing C_{surf} (mol/L), *t* and $R_{(S/L)}$, respectively.

$$[carotenoids] = 1.17 + 0.01C_{surf} + 0.22C_{surf}^2 + 0.12\,t - 0.26t^2 + 0.48R_{(S/L)} + 0.22R_{(S/L)}{}^2 + 0.6C_{surf} \times t + 0.03\,C_{surf} \times R_{(S/L)} + 0.09t \times R_{(S/L)}$$

Equation A2. Polynomial equation obtained for the 2^3 factorial planning carried for Tomadol 25-7, with x, y and z representing C_{surf} (mol/L), *t* and $R_{(S/L)}$, respectively.

$$[carotenoids] = 0.65 + 0.17C_{surf} - 0.05\,C_{surf}^2 + 0.02t + 0.44\,R_{(S/L)} + 0.01R_{(S/L)}{}^2 + 0.01\,C_{surf} \times t + 0.09C_{surf} \times R_{(S/L)} + 0.07\,t \times R_{(S/L)}$$

Equation A3. Polynomial equation obtained for the 2^2 factorial planning carried for Tomadol 25-7, with X and Y representing C_{surf} (mol/L) and $R_{(S/L)}$, respectively.

$$[carotenoids] = 0.65 + 0.17C_{surf} - 0.05C_{surf}{}^2 + 0.44R_{(S/L)} - 0.01R_{(S/L)}{}^2 + 0.09C_{surf} \times R_{(S/L)}$$

1

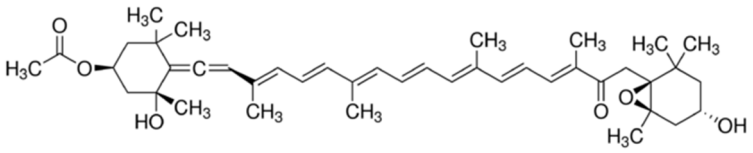


Figure A1. Chemical structure of fucoxanthin.

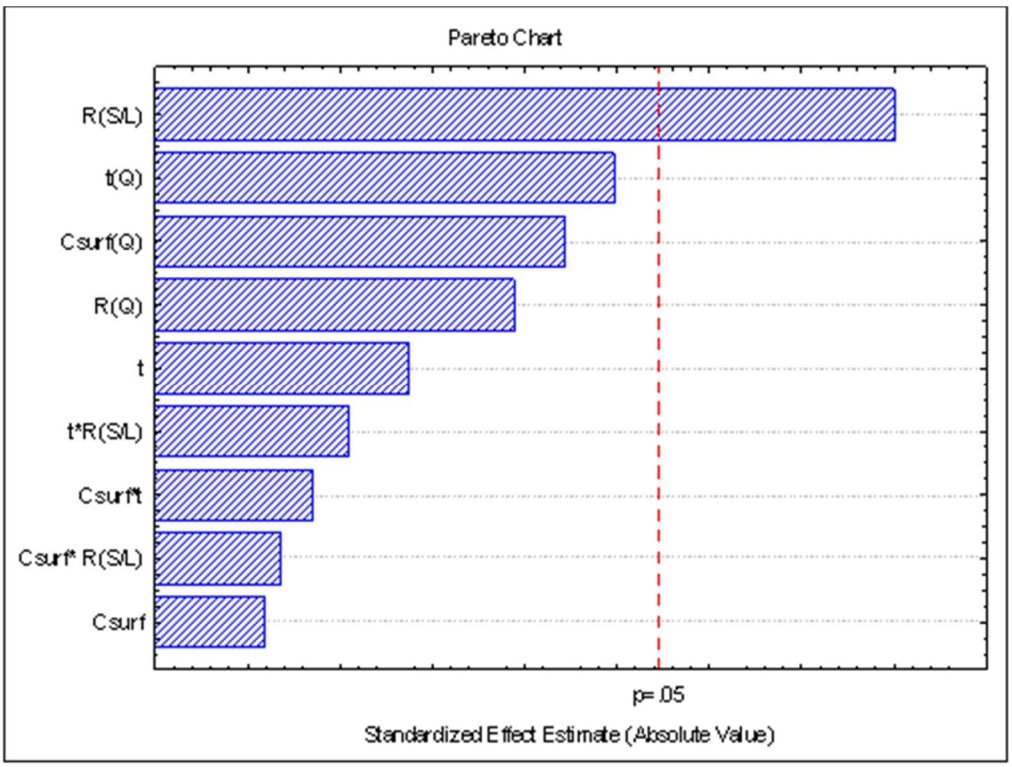


Figure A2. Pareto Chart for Pluronic P-123 experimental design.

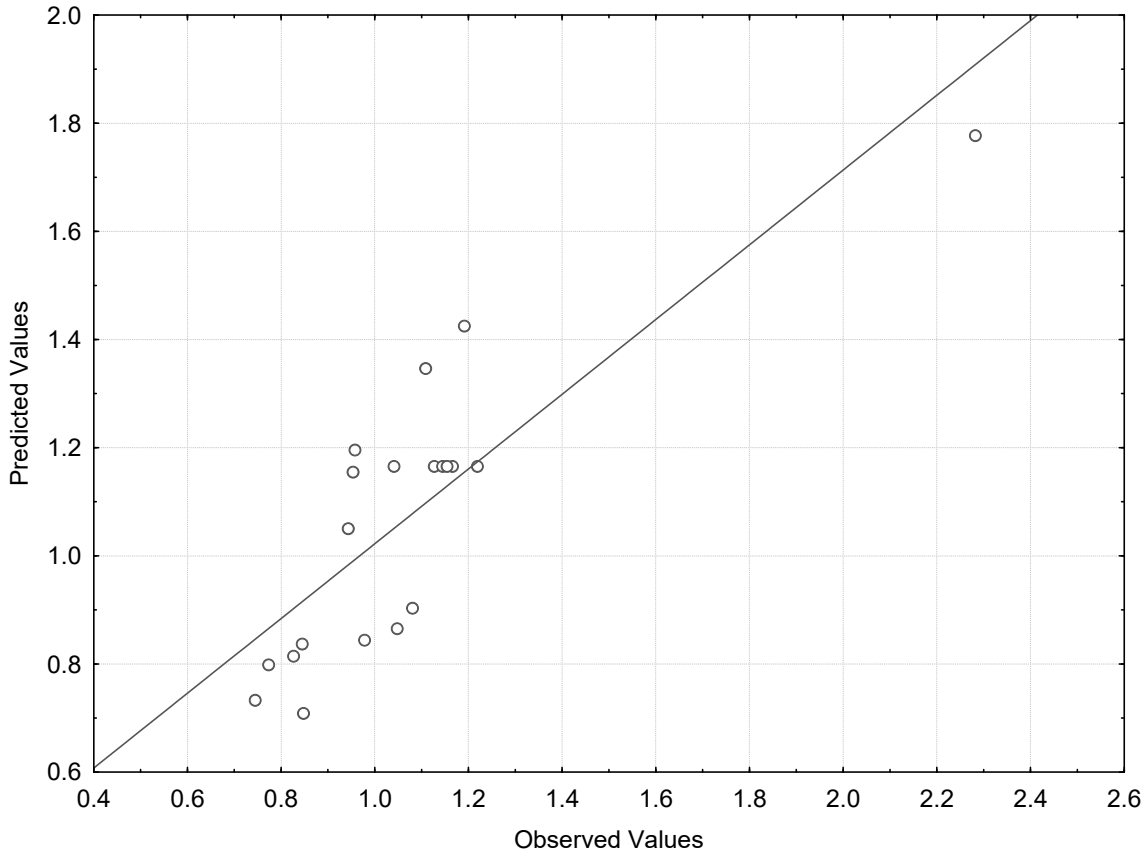


Figure A3. Graphical representation of the amount of carotenoids experimentally extracted and predicted by the model for Pluronic P-123.

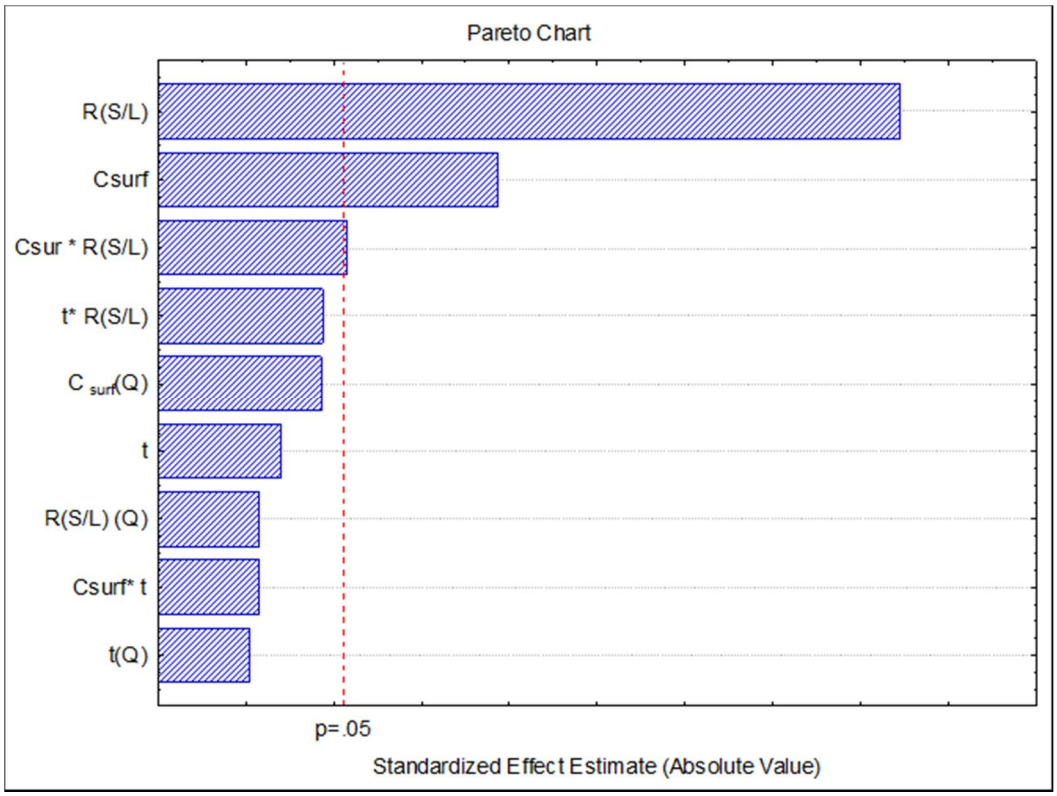


Figure A4. Pareto Chart for Tomadol 25-7 experimental design.

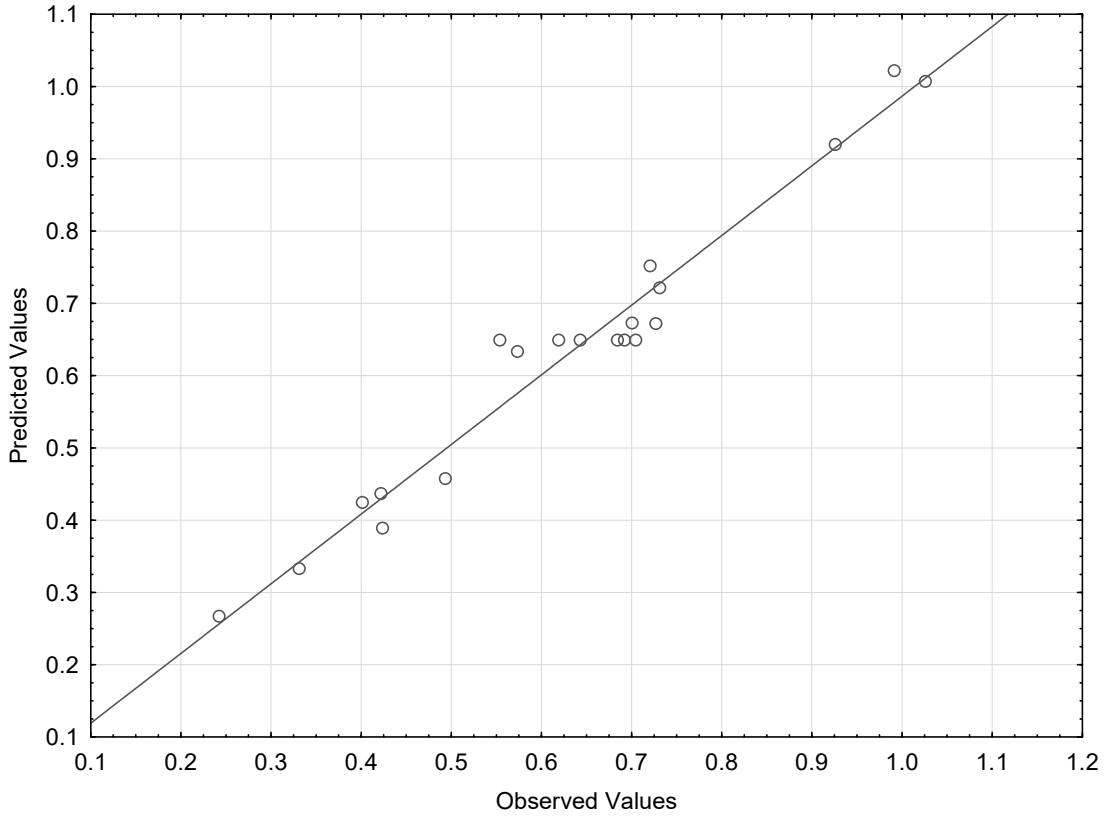


Figure A5. Graphical representation of the amount of carotenoids experimentally extracted and predicted by the model for Tomadol 25-7.

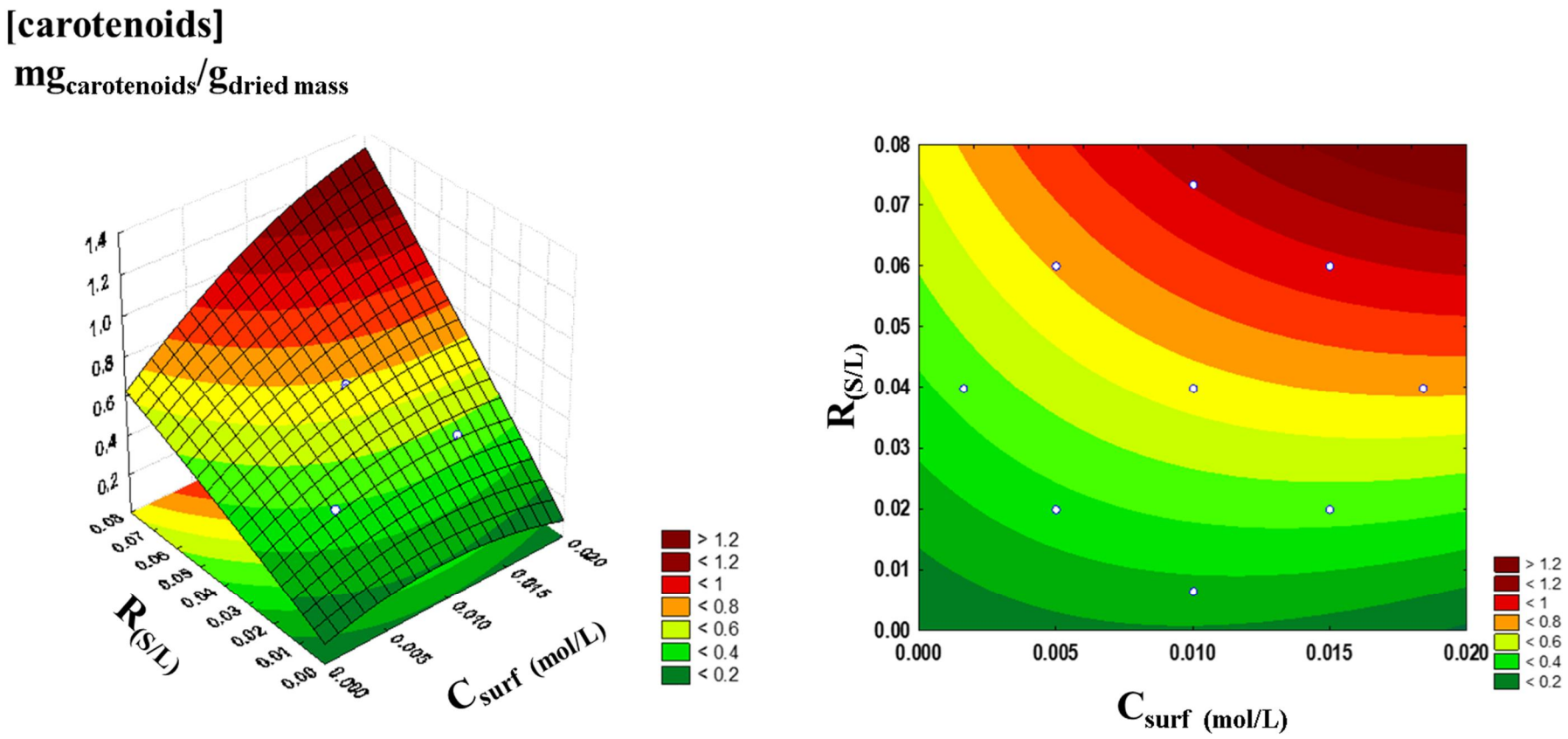


Figure A5. Response surface plot (left) and contour plot (right) on the content of carotenoids ($mg_{carotenoids}/g_{dried\ mass}$) extracted with the combined effects of C_{surf} (mol/L) and $R_{(S/L)}$, using aqueous solutions of Tomadol 25-7 for the new 2^2 factorial planning after the exclusion of the time as a variable.

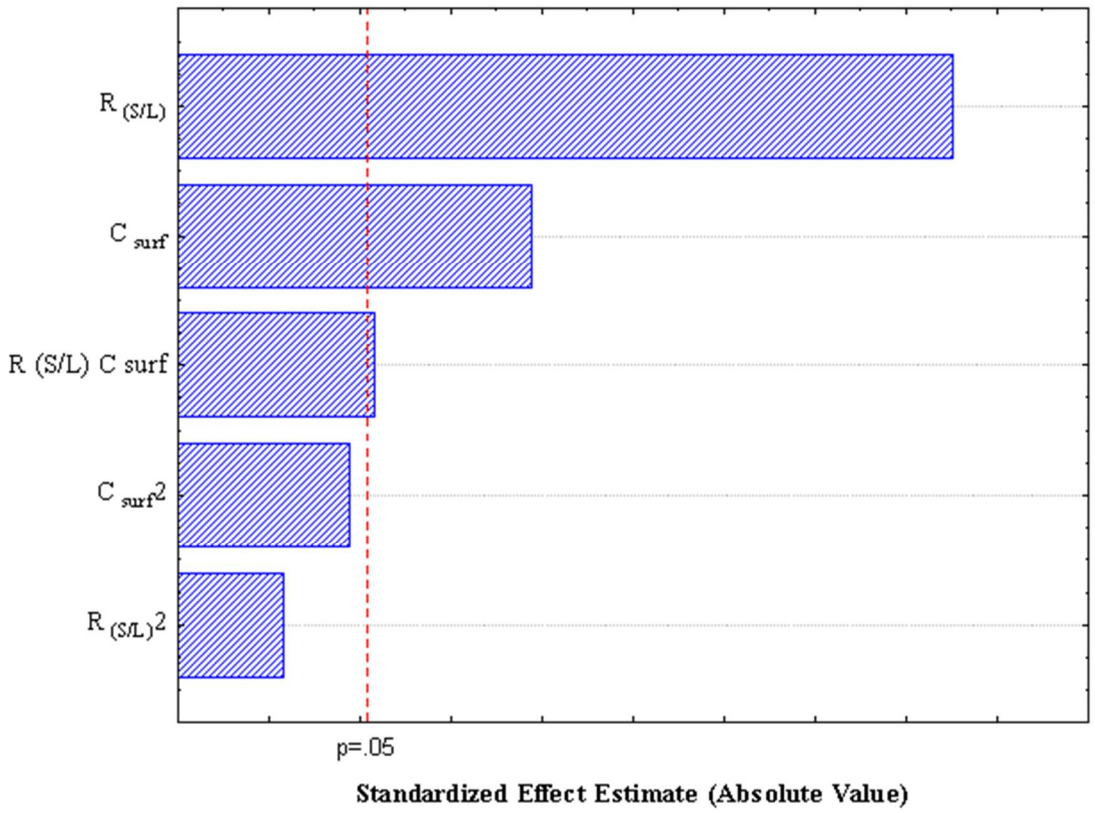


Figure A6. Pareto chart for the 2^2 factorial planning applied to Tomadol 25-7.

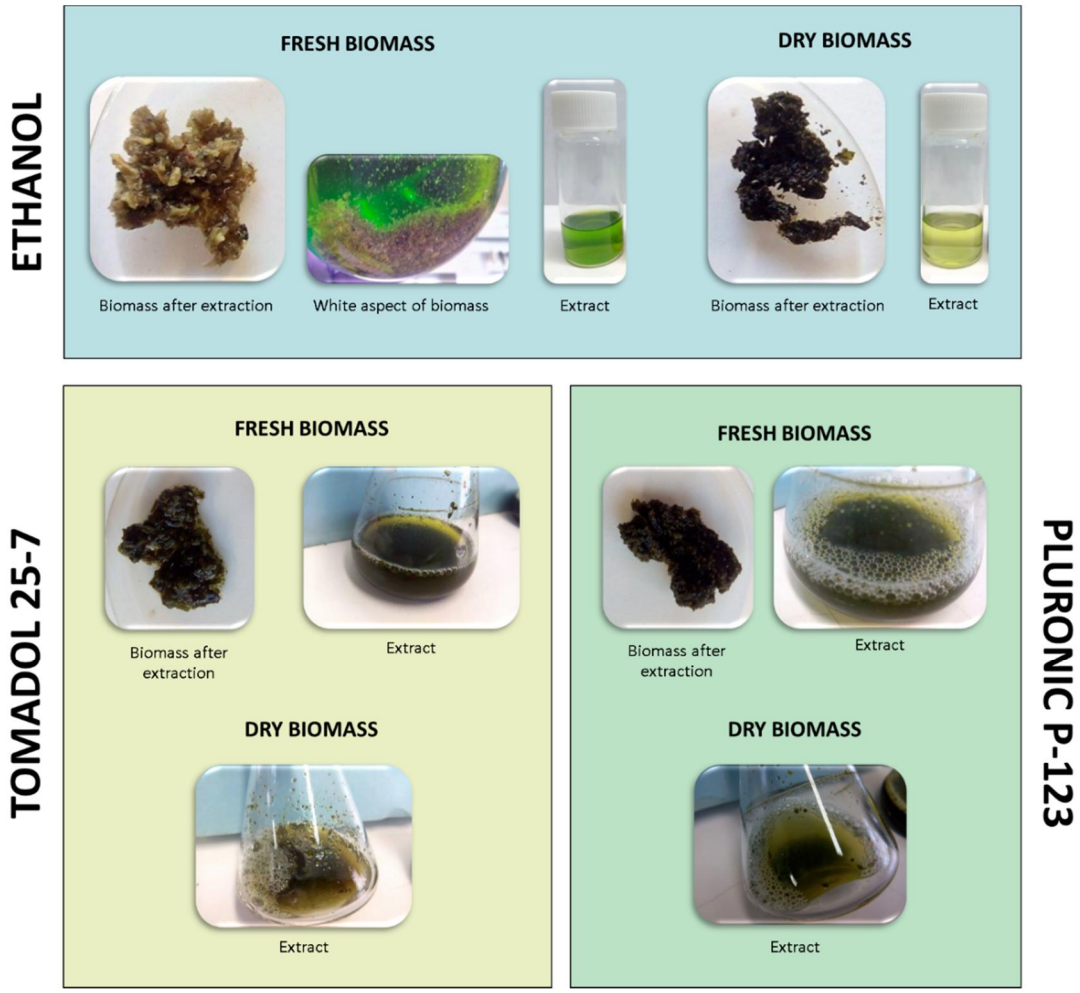


Figure A7. Photos of the biomass after extraction of carotenoids regarding the dry and fresh algal biomass and the different solvents used in this work, ethanol, Tomadol 25-7 and Pluronic P-123.