Electronic Supplementary Material (ESI) for Sustainable Food Technology.

Optimization of *Pavlova gyrans* biomass production and fatty acids profile using a two-step approach

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Supplementary information

ESI Table S1: the twenty-seven experiments combination of the RCCD with the real and coded values (within parentheses) of the independent variables: light intensity (µmol.photons.m^{-2}.s^{-1}), $NaNO_3$ (mg.L^{-1}), $NaH_2PO_4.H_2O$ (mg.L^{-1}) and $CuSO_4.5H_2O$ (µg.L^{-1})

#E	Light intensity (x_3)	$NaNO_3$ (x_5)	$NaH_2PO_4.H_2O$ (x_6)	$CuSO_4.5H_2O$ (x_{15})
1	350 (-1)	750 (-1)	20 (-1)	5 (-1)
2	650 (1)	750 (-1)	20 (-1)	5 (-1)
3	350 (-1)	1250 (1)	20 (-1)	5 (-1)
4	650 (1)	1250 (1)	20 (-1)	5 (-1)
5	350 (-1)	750 (-1)	40 (1)	5 (-1)
6	650 (1)	750 (-1)	40 (1)	5 (-1)
7	350 (-1)	1250 (1)	40 (1)	5 (-1)
8	650 (1)	1250 (1)	40 (1)	5 (-1)
9	350 (-1)	750 (-1)	20 (-1)	15 (1)
10	650 (1)	750 (-1)	20 (-1)	15 (1)
11	350 (-1)	1250 (1)	20 (-1)	15 (1)
12	650 (1)	1250 (1)	20 (-1)	15 (1)
13	350 (-1)	750 (-1)	40 (1)	15 (1)
14	650 (1)	750 (-1)	40 (1)	15 (1)
15	350 (-1)	1250 (1)	40 (1)	15 (1)
16	650 (1)	1250 (1)	40 (1)	15 (1)
17	200 (-2)	1000 (0)	30 (0)	10 (0)
18	800 (2)	1000 (0)	30 (0)	10 (0)
19	500 (0)	500 (-2)	30 (0)	10 (0)
20	500 (0)	1500 (2)	30 (0)	10 (0)
21	500 (0)	1000 (0)	10 (-2)	10 (0)
22	500 (0)	1000 (0)	50 (2)	10 (0)
23	500 (0)	1000 (0)	30 (0)	0 (-2)
24	500 (0)	1000 (0)	30 (0)	20 (2)
25	500 (0)	1000 (0)	30 (0)	10 (0)
26	500 (0)	1000 (0)	30 (0)	10 (0)
27	500 (0)	1000 (0)	30 (0)	10 (0)

ESI Table S2: combination of growth conditions used in the validation experiments: optimized conditions, Opt, control/Walne's medium, Con, medium without vitamins, Vit-, and assay with the non-significant variables of the PB design defined at Level -1, Lvl-1

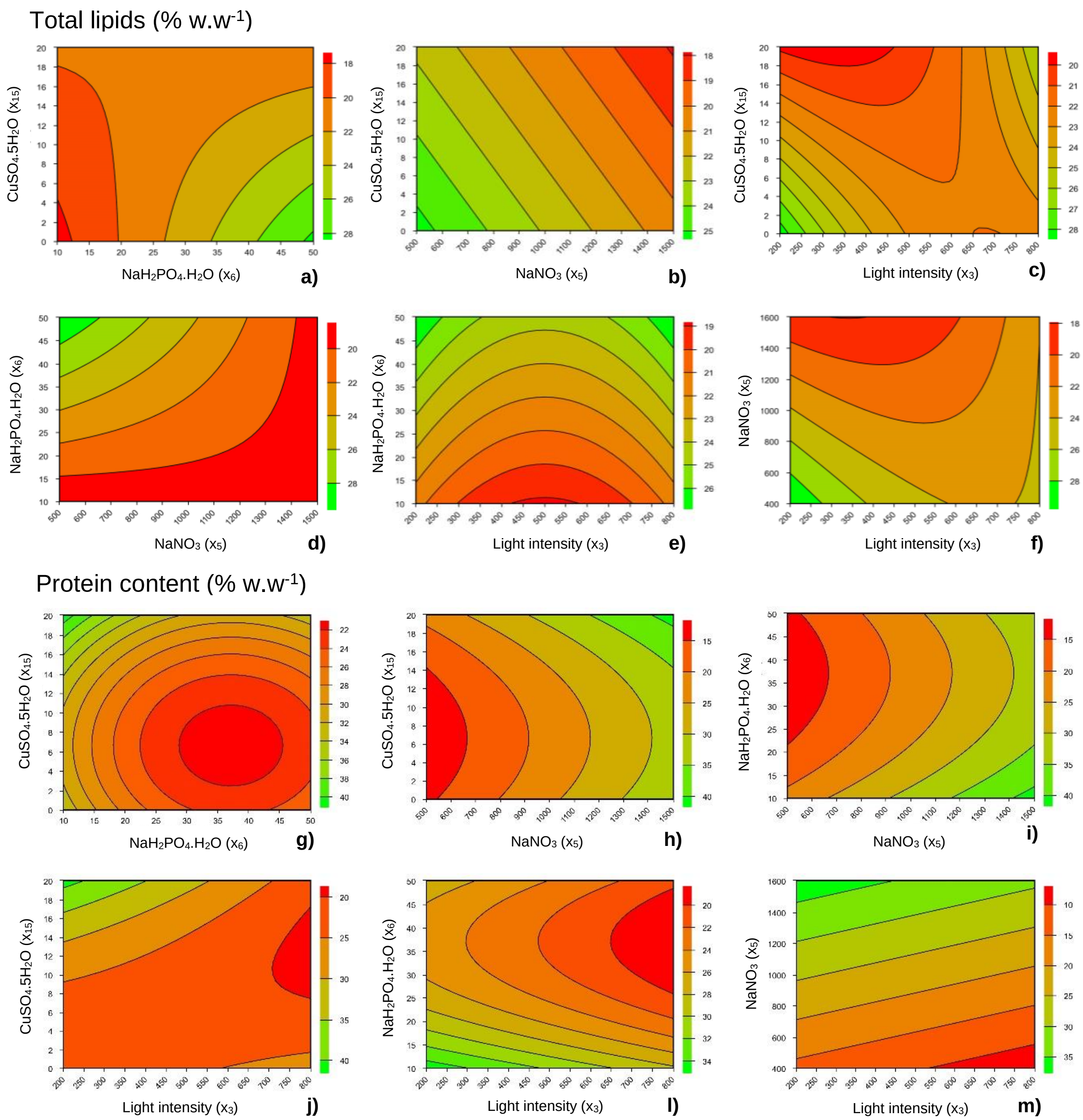
Variable	Opt	Lvl-1	Vit-	Con
Light intensity (µmol.photons.$m^{-2}.s^{-1}$)	700	700	700	700
$NaNO_3$ (mg.L^{-1})	1500	1500	1500	100
$CuSO_4.5H_2O$ (µg.L^{-1})	6	6	6	20 (0)
$NaH_2PO_4.H_2O$ (mg.L^{-1})	40	40	40	20 (0)
$Na_2H_2EDTA.2H_2O$ (mg.L^{-1})	45 (0)	22.5 (-1)	45 (0)	45 (0)
H_3BO_3 (mg.L^{-1})	33.6 (0)	16.8 (-1)	33.6 (0)	33.6 (0)
$FeCl_3.6H_2O$ (mg.L^{-1})	1.3 (0)	0.65 (-1)	1.3 (0)	1.3 (0)
$MnCl_2.4H_2O$ (µg.L^{-1})	360 (0)	180 (-1)	360 (0)	360 (0)
$ZnCl_2$ (µg.L^{-1})	21 (0)	10.25 (-1)	21 (0)	21 (0)
$CoCl_2.6H_2O$ (µg.L-^{1})	20 (0)	10 (-1)	20 (0)	20 (0)
$(NH_4)_6Mo_7O_{24}.4H_2O$ (µg.L^{-1})	9 (0)	4.5 (-1)	9 (0)	9 (0)
Thiamine (µg.L^{-1})	100 (0)	50 (-1)	-	100 (0)
Cyanocobalamin (µg.L^{-1})	5 (0)	2.5 (-1)	-	5 (0)
$NaHCO_3$ (mg.L^{-1})	170 (-1)	170 (-1)	170 (-1)	-
Salinity (psu)	30 (0)	20 (-1)	30 (0)	30 (0)
Air flow (mL.min^{-1})	600 (-1)	600 (-1)	600 (-1)	600 (-1)
Inoculum size (g AFDW.L^{-1})	0.1 (-1)	0.1 (-1)	0.1 (-1)	0.1 (-1)

ESI Table S3: values of maximum biomass produced (X_{max}, g AFDW.L^{-1}) and volumetric biomass productivity (P_X, g AFDW.L^{-1}.d^{-1}) at the beginning of the stationary phase (t_x, days) for the *P. gyrans* grown in the validation assays: optimized conditions, Opt, control/Walne's medium, Con, medium without vitamins, Vit-, and assay with the variables considered non-significant in PB design fixed at Level -1, Lvl-1. Values are the mean and standard deviation of three replicates (n=3). Different letters indicate significant differences between the validation assays (one-way ANOVA, $p < 0.05$, followed by the Tukey's test). Gain was calculated as the ratio of $X_{max}/X_{max.control}$

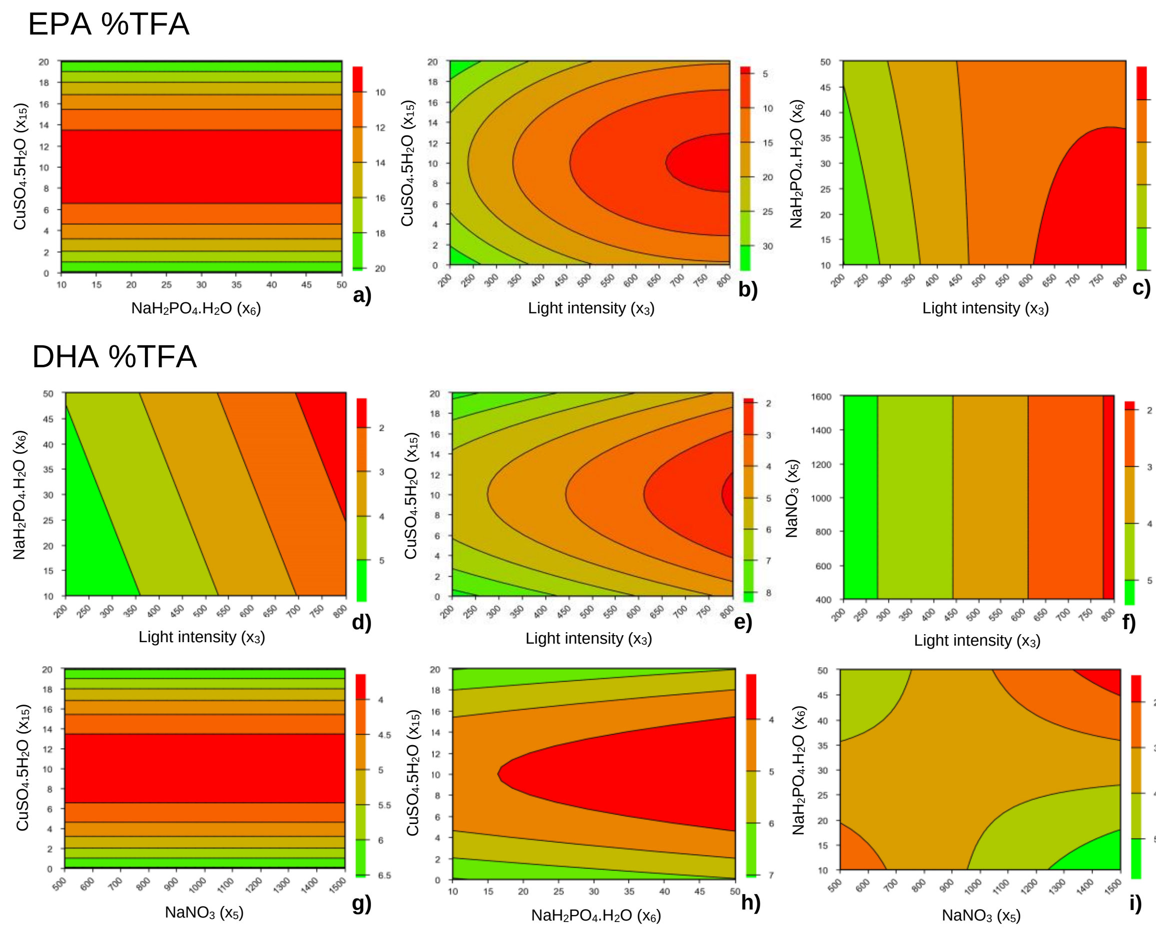
	t_x (d)	P_x	X_{max}	Gain
Con	4	0.148 ± 0.003^{A}	0.59 ± 0.01^{a}	1.0
Lvl-1	4	0.229 ± 0.013^{B}	0.92 ± 0.05^{b}	1.5
Vit-	4	0.153 ± 0.001^{A}	0.61 ± 0.01^{a}	1.0
Opt	10	0.225 ± 0.005^{B}	2.26 ± 0.05^{c}	3.8

ESI Table S4: experimental and predicted values, as well as the relative errors *(%RE* = 100 x *(Exp-Pred)/Exp*), for the responses maximum biomass produced (Xmax, g AFDW.L^{-1}), protein content (% w.w^{-1}), total lipids (% w.w^{-1}), eicosapentaenoic acid (EPA, %TFA) and docosahexaenoic acid (DHA, %TFA), achieved under the optimal conditions defined for validation of the mathematical models produced

Response	Experimental	Predicted	%RE
X_{max}	2.26 ± 0.05	2.34	-3.69
Protein content	30.76 ± 4.37	30.58	0.60
Total lipids	28.30 ± 0.95	22.05	22.10
EPA	20.69 ± 1.61	7.37	64.39
DHA	10.33 ± 0.30	1.80	82.54



ESI Figure S1: Contour curves from RCCD for the dependent variable total lipids (% w.w^{-1}), a-f), illustrating the interactions between $CuSO_4.5H_2O$ and $NaH_2PO_4.H_2O$, (a), $CuSO_4.5H_2O$ and $NaNO_3$, (b), $CuSO_4.5H_2O$ and light intensity, (c), $NaH_2PO_4.H_2O$ and $NaNO_3$, (d), $NaH_2PO_4.H_2O$ and light intensity, (e), and $NaNO_3$ and light intensity, (f). Contour curves for protein content (% w.w^{-1}), g-m), illustrating the interactions between $CuSO_4.5H_2O$ and $NaH_2PO_4.H_2O$, (g), $CuSO_4.5H_2O$ and $NaNO_3$, (h), $NaH_2PO_4.H_2O$ and $NaNO_3$, (i), $CuSO_4.5H_2O$ and light intensity, (j), $NaH_2PO_4.H_2O$ and light intensity, l), $NaNO_3$ and light intensity, m)



ESI Figure S2: Contour curves from RCCD for the dependent variable EPA %TFA, a-c), illustrating the interactions between $CuSO_4.5H_2O$ and $NaH_2PO_4.H_2O$, a), $CuSO_4.5H_2O$ and light intensity, b), and $NaH_2PO_4.H_2O$ and light intensity, c). Contour curves for DHA %TFA, d-i), illustrating the interactions between $NaH_2PO_4.H_2O$ and light intensity, d), $CuSO_4.5H_2O$ and light intensity, e), $NaNO_3$ and light intensity, f), $CuSO_4.5H_2O$ and $NaNO_3$, (h), $CuSO_4.5H_2O$ and $NaH_2PO_4.H_2O$, h), and $NaH_2PO_4.H_2O$ and $NaNO_3$, i)