## Supplementary Material

*Article*

# Comparative Study of Conventional, Microwave Assisted and Supercritical Fluid Extraction of Bioactive Compounds from Microalgae: The Case of *Scenedesmus obliquus*

**Ioulia Georgiopoulou *, Vasiliki Louli and Kostis Magoulas ***

Laboratory of Thermodynamics and Transport Phenomena, School of Chemical Engineering, National Technical University of Athens (NTUA), Zografou Campus, Athens 15780, Greece;
svlouli@chemeng.ntua.gr (V.L.)
* Correspondence: iouliageo@mail.ntua.gr (I.G.); mag@chemeng.ntua.gr (K.M.), Tel.: +30-210-772-3230 (I.G.); +30-210-772-3152 (K.M.)

Modelling & Optimization

All the equations were chosen under the assumption that models are statistically significant and provide successful fitting, and hierarchy of terms is maintained.

S1. SLE – ANOVA

The ANOVA results, regarding SLE data, are presented below. Equation 1 was applied for data fitting, whereby all the responses were successfully correlated, except from the selected carotenoid content. All the models are presented below (equations S1-S5).

$$Yield = -8.6324 + 0.3825\,T + 0.0976\,t + 0{,}0738\,R \quad \text{(S1)}$$

$$TPC = 9.8802 + 0.1478\,T + 0.7942\,t + 0.0283\,R - 0.0175\,Tt \quad \text{(S2)}$$

$$CHL = 29.3921 + 1.1645\,T + 5.1768\,t - 0.0891\,R - 0.1172\,Tt \quad \text{(S3)}$$

$$CAR = 14.5966 - 0.0102\,T + 0.3662\,t - 7.9791\,10^{-3}\,Tt \quad \text{(S4)}$$

$$IC_{50} = 78.8509 - 0.5379\,T - 2.4935\,t + 0.0564\,Tt \quad \text{(S5)}$$

The Pareto charts presented in Figure S1 prove that the statistically significant parameters at a level of $p$-values of F-test lower than 0.05 and above the $t$-limit are temperature ($p$<0.0001) and solvent-to-biomass ratio ($p$=0.0002) for yield, the temperature and time interaction ($p$=0.0021) and temperature ($p$=0.0080) for total phenolic content, the temperature and time interaction ($p$=0.0004) and temperature ($p$=0.0056) for chlorophyll content, temperature ($p$=0.0022) for carotenoid content, and the temperature and time interaction ($p$=0.0190) for extract's antioxidant activity. Moreover, extraction temperature is the most statistically significant positive effect on yield and negative on extract's carotenoid content, while the combined effect of extraction temperature and duration ($Tt$) proved to be the most significant positive effect on phenolic and chlorophyll content, and $IC_{50}$ value, which indicates a negative effect on extract's antioxidant activity.

Based on the ANOVA results presented in Table S1, $F$-tests' $p$-values prove the statistical significance of the models ($p$<0.05) and insignificance of the lack of fit ($p$>0.1). The high values of the coefficient of determination ($R^2$) as well as the affinity of the experimental and predicted data, as shown in Figure S2, indicate the satisfactory models' precision. Finally, the considerable values of adequate precision (Ad. Prec.>4) prove the accuracy of the referred models.

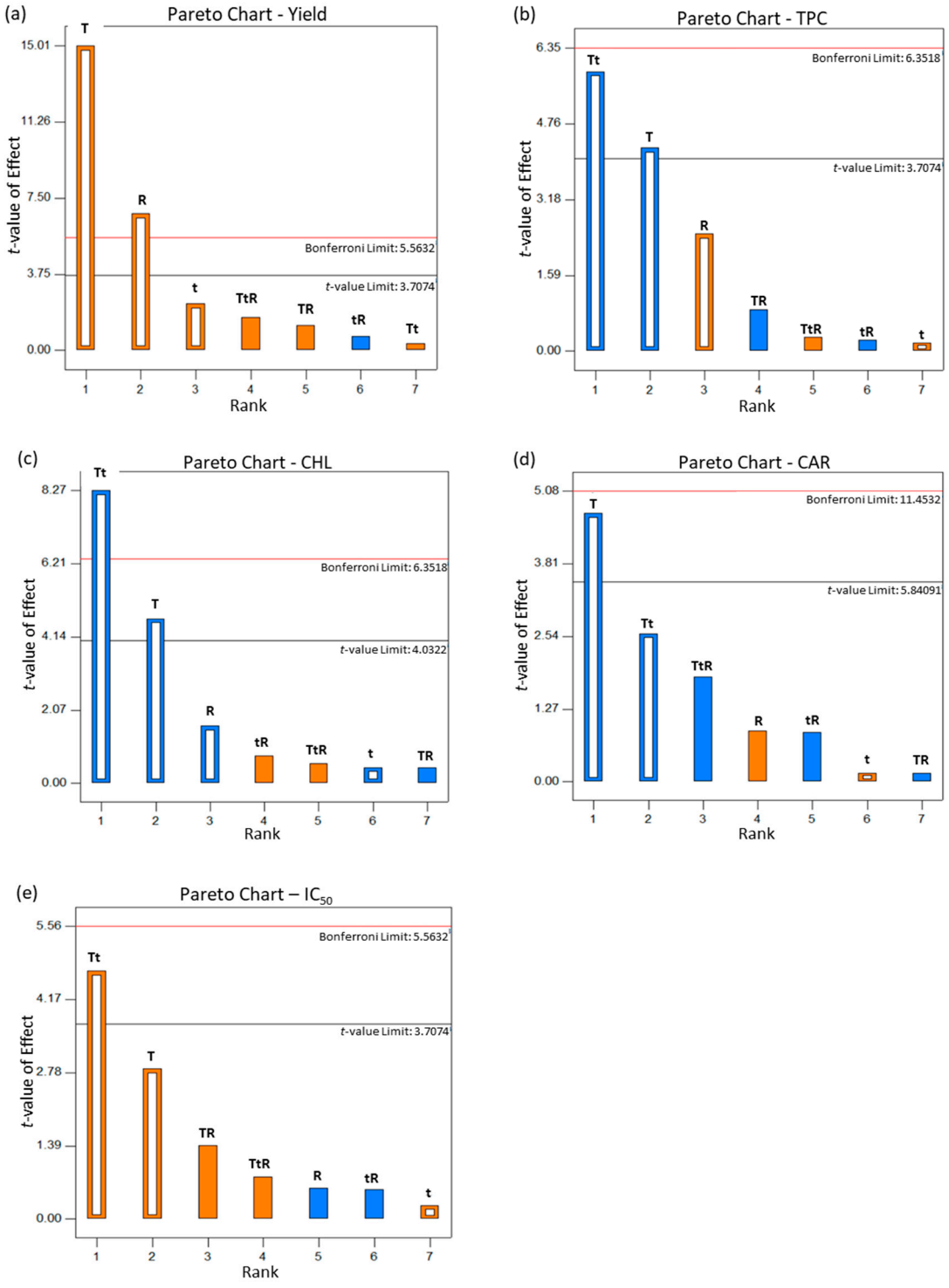


Figure S1. The Pareto chart for the analysis of SLE's **(a)** yield, and extract's total **(b)** phenolic, **(c)** chlorophyll and **(d)** carotenoid content, and **(e)** index of antioxidant activity. Orange and blue columns indicate positive and negative effects, respectively.

Table S1. The main ANOVA results and adequacy measures of the successfully correlated responses examined for the SLE of *S. obliquus*.

| Yield | | | |
|---|---|---|---|
| Source | *F*-value | *p*-value | |
| Model | 92.07 | < 0.0001 | significant |
| Lack of Fit | 2.06 | 0.3530 | not significant |
| $R^2$ | 0.9787 | Ad. Prec. | 25.24 |
| TPC | | | |
| Source | *F*-value | *p*-value | |
| Model | 14.64 | 0.0057 | significant |
| Lack of Fit | 0.15 | 0.9234 | not significant |
| $R^2$ | 0.9213 | Ad. Prec. | 14.36 |
| CHL | | | |
| Source | *F*-value | *p*-value | |
| Model | 23.25 | 0.0020 | significant |
| Lack of Fit | 0.19 | 0.8969 | not significant |
| $R^2$ | 0.9490 | Ad. Prec. | 13.94 |
| CAR | | | |
| Source | *F*-value | *p*-value | |
| Model | 8.24 | 0.0151 | not significant |
| Lack of Fit | 1.22 | 0.4959 | not significant |
| $R^2$ | 0.8047 | Ad. Prec. | 7.08 |
| $IC_{50}$ | | | |
| Source | *F*-value | *p*-value | |
| Model | 10.15 | 0.0091 | significant |
| Lack of Fit | 0.60 | 0.7016 | not significant |
| $R^2$ | 0.8354 | Ad. Prec. | 7.94 |

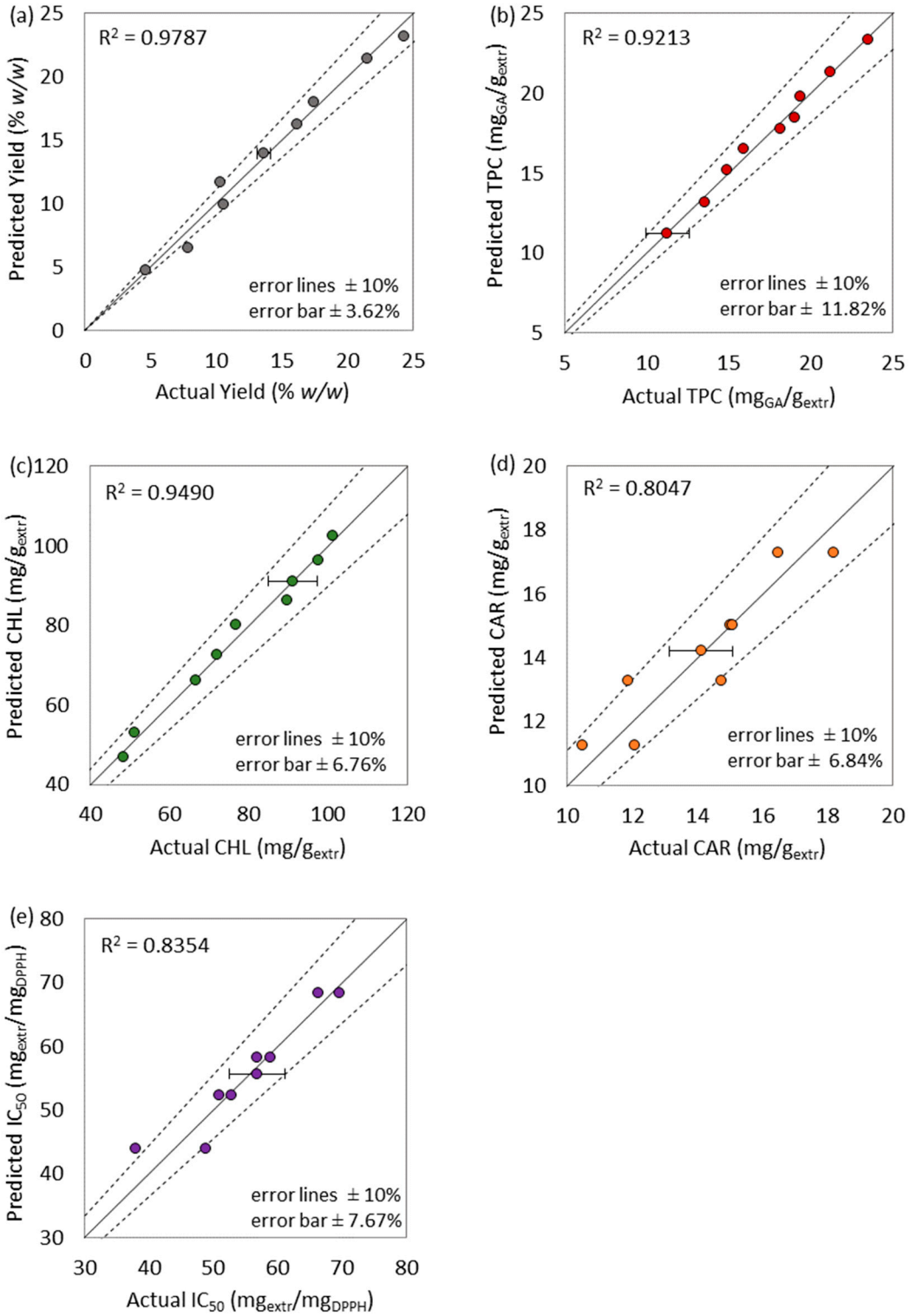


Figure S2. Experimental versus predicted values of SLE's **(a)** yield, and extract's total **(b)** phenolic, **(c)** chlorophyll and **(d)** carotenoid content, and **(e)** index of antioxidant activity. The error bars stands for the experimental error.

S2. MAE – ANOVA

The ANOVA results, regarding MAE data, are presented below. Equation 2 was applied for data fitting, whereby only the responses of yield and extract's antioxidant activity were successfully correlated. The corresponding equations are given below (equations S6 and S7):

$$Yield = -12.4566 + 0.1616\,T + 0.5493\,t + 0.0972\,R + 0.0177\,P + 1.5531\,10^{-3}\,TR - 5.6595\,10^{-3}\,tR - 7.5748\,10^{-3}\,tP - 1.7720\,10^{-3}\,RP + 8.2302\,10^{-6}\,tRP \quad \text{(S6)}$$

$$IC_{50} = 113.7052 - 1.4960\,T + 1.4393\,t - 0.0292\,R - 0.0628\,P - 0.0419\,Tt + 1.3408\,10^{-3}\,TP + 0.0101\,tR - 5.3962\,10^{-3}\,tP + 1.1666\,10^{-4}\,TtP \quad \text{(S7)}$$

The Pareto charts presented in Figure S3 prove that the statistically significant parameters at a level of *p*-values of F-test lower than 0.05 and above the *t*-limit are temperature ($p$<0.0001), solvent-to-biomass ratio ($p$<0.0001) and microwave power ($p$=0.0024) for yield, and the temperature and microwave power interaction ($p$<0.0001), solvent-to-biomass ratio ($p$=0.0040) and temperature ($p$=0.0043) for extract's antioxidant activity. Moreover, extraction temperature is the most statistically significant positive effect on yield, while the combined effect of extraction temperature and microwave power ($TP$) proved to be the most significant positive effect on $IC_{50}$ value, which indicates a negative effect on extract's antioxidant activity.

Based on the ANOVA results presented in Table S2, *F*-tests' *p*-values prove the statistical significance of the models ($p$<0.05) and insignificance of the lack of fit ($p$>0.1). The high values of $R^2$ as well as the affinity of the experimental and predicted data, as shown in Figure S4, indicate the satisfactory models' precision. Finally, the considerable values of adequate precision (Ad. Prec.>4) prove the accuracy of the referred models.

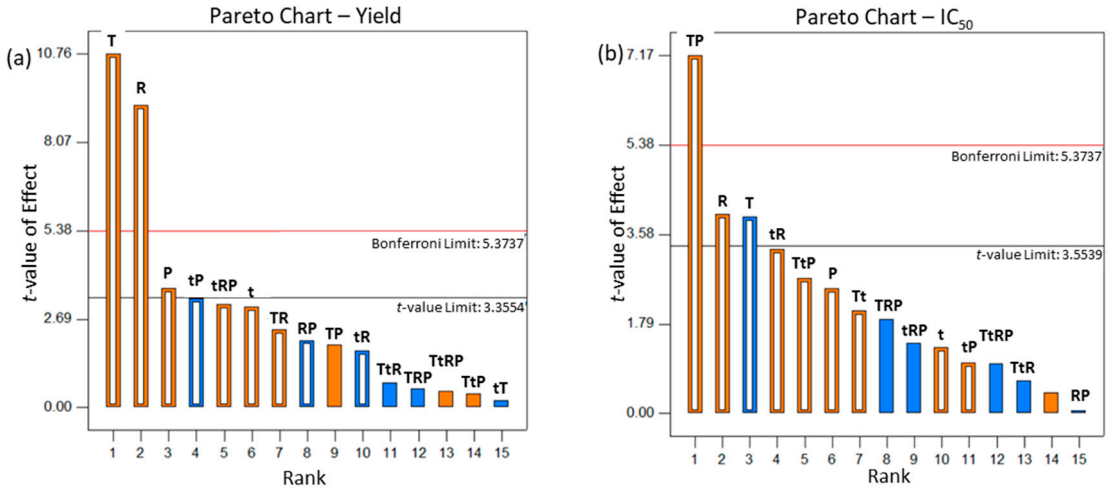


Figure S3. The Pareto chart for the analysis of MAE's **(a)** yield, and **(b)** extract's index of antioxidant activity. Orange and blue columns indicate positive and negative effects, respectively.

Table S2. The main ANOVA results and adequacy measures of the successfully correlated responses examined for the MAE of *S. obliquus*.

| **Yield** | | | |
|---|---|---|---|
| Source | *F*-value | *p*-value | |
| Model | 42.04 | < 0.0001 | significant |
| Lack of Fit | 0.18 | 0.9466 | not significant |
| $R^2$ | 0.9836 | Ad. Prec. | 23.82 |
| **$IC_{50}$** | | | |
| Source | *F*-value | *p*-value | |
| Model | 12,69 | 0.0008 | significant |
| Lack of Fit | 2,77 | 0.2892 | not significant |
| $R^2$ | 0.9345 | Ad. Prec. | 13.58 |

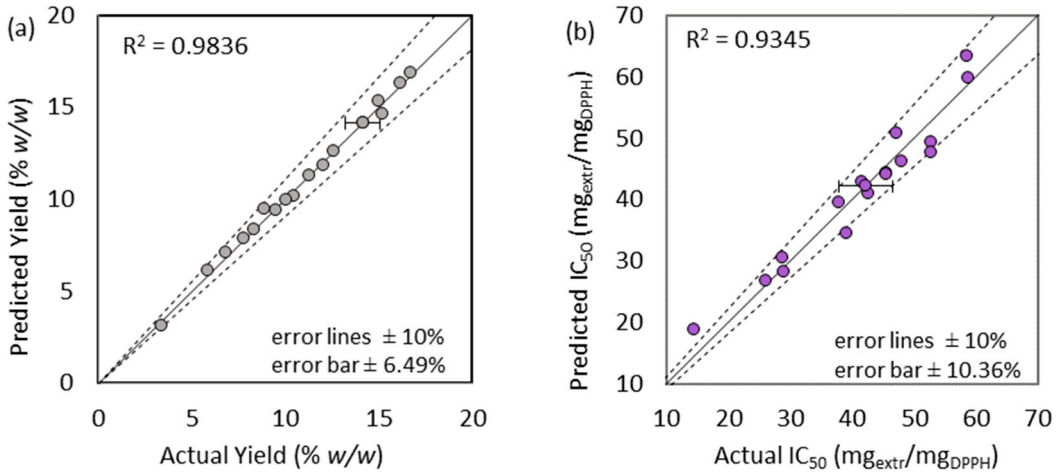


Figure S4. Experimental versus predicted values of MAE's **(a)** yield, and **(b)** extract's index of antioxidant activity. The error bars stands for the experimental error.

S3. SFE – ANOVA

The ANOVA results, regarding SFE data, are presented below. Equation 1 was applied for data fitting, whereby all the responses were successfully correlated. All the models are presented through equations S8-S13.

$$Yield = 0.5337 - 4.0398\,10^{-3}\,T - 0{,}0191\,P + 0.0105\,F + 5.5821\,10^{-4}\,TP \quad \text{(S8)}$$

$$TPC = -8.0493 + 0.0782\,T + 0.0617\,P - 0.0217\,F \quad \text{(S9)}$$

$$CHL = -10.6522 + 0.1820\,T + 0.0641\,P - 7.7685\,F - 4.1879\,10^{-4}\,TP \quad \text{(S10)}$$

$$sel.CAR = -21.4308 + 0.4412\,T + 0.0754\,P - 0.3154\,F + 1.9165\,10^{-3}\,PF \quad \text{(S11)}$$

$$CAR = -35.5629 + 0.5312\,T + 0.2446\,P - 0.5460\,F - 2.0106\,10^{-3}\,TP + 0.0132\,TF \quad \text{(S12)}$$

$$IC_{50} = 230.8782 - 2.8676\,T - 0.5721\,P + 1.6052\,F + 7.1520\,10^{-3}\,TP - 6.2477\,10^{-3}\,PF \quad \text{(S13)}$$

The Pareto charts presented in Figure S5 prove that the statistically significant parameters at a level of $p$-values of F-test lower than 0.05 and above the $t$-limit are temperature ($p$<0.0001) pressure ($p$<0.0001) and the interaction between temperature and pressure ($p$=0.0007) for yield, pressure ($p$<0.0001) and temperature ($p$=0.0017) for selected carotenoid content, and pressure ($p$<0.0001) and temperature ($p$=0.0003) for extract's antioxidant activity. The extraction pressure was considered statistically significant for total phenolic ($p$=0.0042), chlorophyll ($p$=0.0007) and carotenoid ($p$=0.0014) content. The extraction temperature is considered the most statistically significant positive effect on yield. Moreover, pressure proved to be the most statistically significant positive effect on phenolic, chlorophyll and carotenoid content, but also the most statistically significant negative effect on the $IC_{50}$ value, which indicates a positive effect on extract's antioxidant activity.

Based on the ANOVA results presented in Table S3, $F$-tests' $p$-values prove the statistical significance of the models ($p$<0.05) and insignificance of the lack of fit ($p$>0.1). The high values of $R^2$ as well as the affinity of the experimental and predicted data presented, as shown in Figure S6, indicate the satisfactory models' precision. Finally, the considerable values of adequate precision (Ad. Prec.>4) prove the accuracy of the referred models.

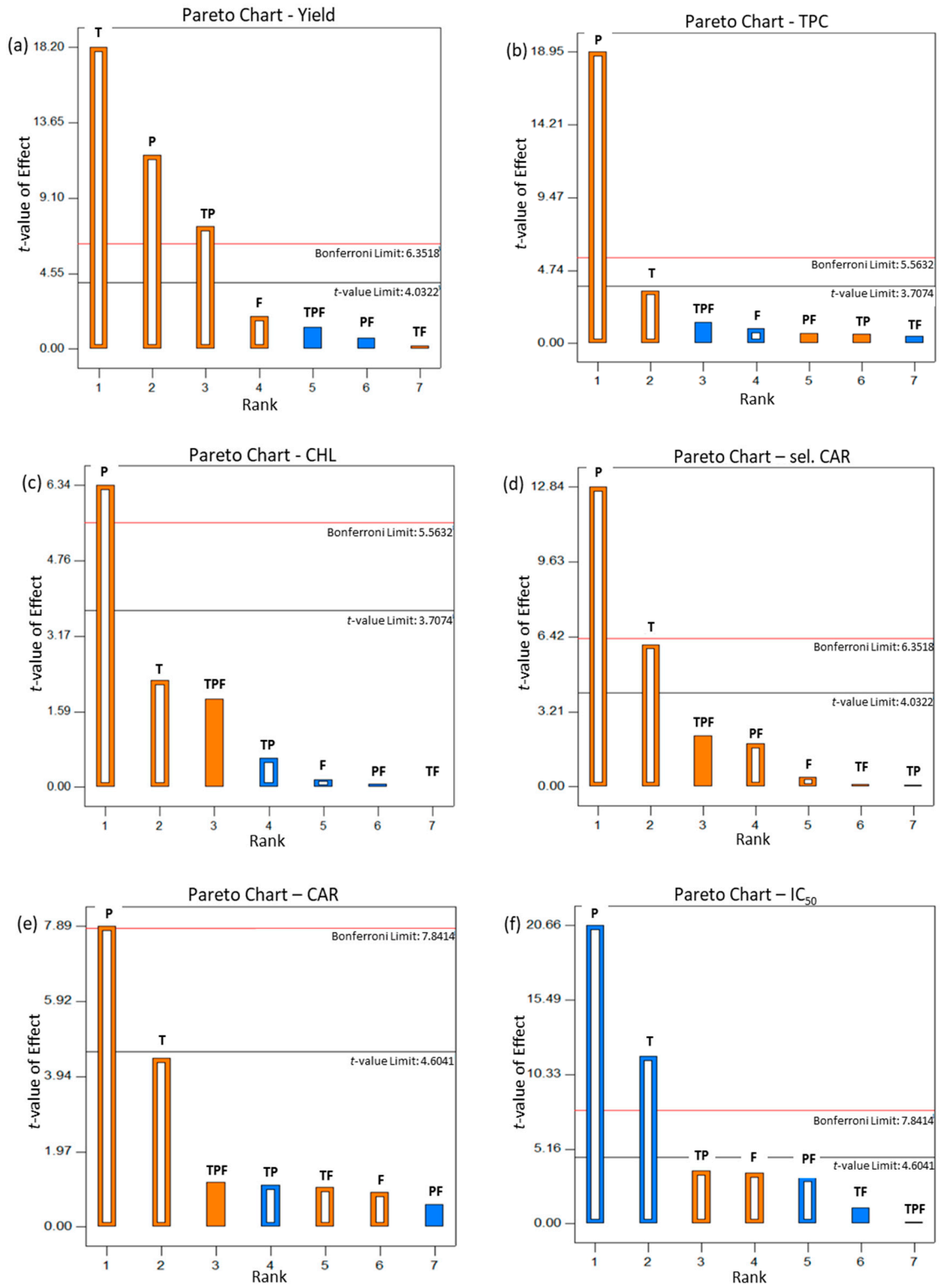


Figure S5. The Pareto chart for the analysis of SFE's **(a)** yield, and extract's **(b)** phenolic, **(c)** chlorophyll content, **(d)** selected and **(e)** total carotenoid content, and **(f)** index of antioxidant activity. Orange and blue columns indicate positive and negative effects, respectively.

Table S3. The main ANOVA results and adequacy measures of the successfully correlated responses examined for the SFE of *S. obliquus*.

| **Yield** | | | |
|---|---|---|---|
| Source | *F*-value | *p*-value | |
| Model | 131.48 | < 0.0001 | significant |
| Lack of Fit | 0.52 | 0.7087 | not significant |
| $R^2$ | 0.9906 | Ad. Prec. | 30.50 |
| **TPC** | | | |
| Source | *F*-value | *p*-value | |
| Model | 123.91 | < 0.0001 | significant |
| Lack of Fit | 0.50 | 0.7519 | not significant |
| $R^2$ | 0.9841 | Ad. Prec. | 24.47 |
| **CHL** | | | |
| Source | *F*-value | *p*-value | |
| Model | 13.36 | 0.0070 | significant |
| Lack of Fit | 2.69 | 0.2825 | not significant |
| $R^2$ | 0.9144 | Ad. Prec. | 9.06 |
| **sel. CAR** | | | |
| Source | *F*-value | *p*-value | |
| Model | 51.41 | 0.0003 | significant |
| Lack of Fit | 13.97 | 0.0675 | not significant |
| $R^2$ | 0.9763 | Ad. Prec. | 19.90 |
| **CAR** | | | |
| Source | *F*-value | *p*-value | |
| Model | 16.99 | 0.0085 | significant |
| Lack of Fit | 0.76 | 0.5668 | not significant |
| $R^2$ | 0.9550 | Ad. Prec. | 11.84 |
| **$IC_{50}$** | | | |
| Source | *F*-value | *p*-value | |
| Model | 119.51 | 0.0002 | significant |
| Lack of Fit | 0.43 | 0.6994 | not significant |
| $R^2$ | 0.9934 | Ad. Prec. | 31.72 |

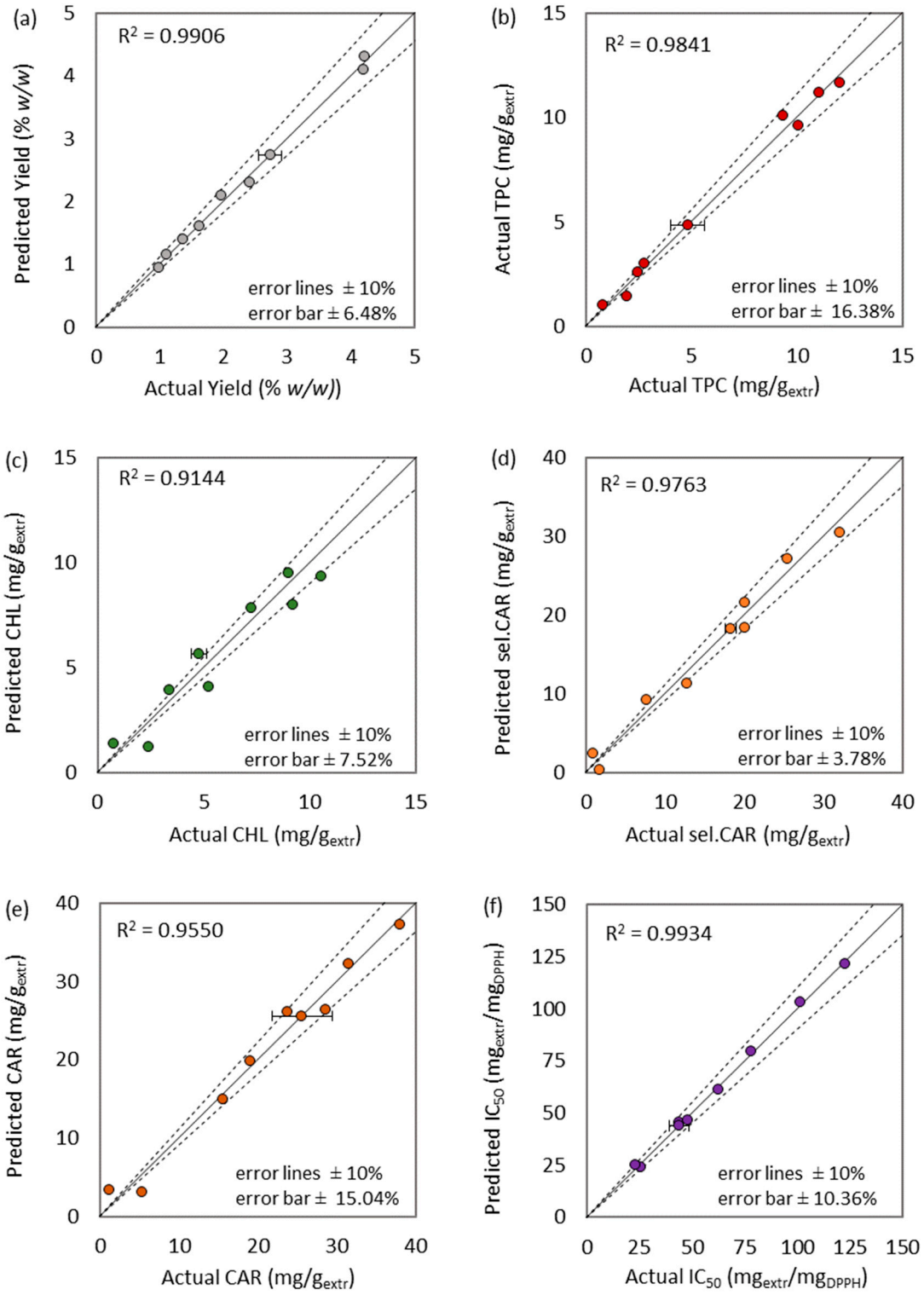


Figure S6. Experimental versus predicted values of SFE's **(a)** yield, and extract's **(b)** phenolic, **(c)** chlorophyll, **(d)** selected and **(e)** total carotenoid content, and **(f)** index of antioxidant activity. The error bars stands for the experimental error.