Peptone 1: peptone from pea; Peptone 2: wheat peptone; Peptone 3: potato peptone).

[105] The experimental design for OD_{540nm} values according to the proportion of plant peptones obtained through custom design is shown in Table 6 below, and the toxin concentration after the completion of culture under each condition was measured and added to the analysis items (Peptone 1: peptone from pea; Peptone 2: wheat peptone; Peptone 3: potato peptone).

[106] [Table 6]

	Peptone 1	Peptone 2	Peptone 3	OD540nm
1	10	20	0	
2	10	0	10	
3	20	10	0	
4	10	0	0	
5	10	10	20	
6	0	10	0	
7	0	0	0	
8	20	20	0	
9	0	20	20	
10	0	20	10	
11	20	20	20	
12	20	0	0	
13	20	0	20	
14	0	20	0	
15	20	10	10	
16	0	0	20	

[107] 2-2: Compositions of APF media used for culture

[108] As shown in Table 7 below, to 1% glucose [10 g/L] as a carbon source and 1% yeast extract [10 g/L], 2% three plant peptones [10 g/L] were added. Each of the mixtures was dissolved in 90 mL of water for injection, and the final volume was adjusted to 100 mL. 10-mL aliquots of the prepared

[117] [Table 9]

	Pea	Wheat	Potato	OD at 24hr	Toxin at 48hr
1	10	20	0	6.0792	42041.2
2	10	0	10	3.1707	56296.2
3	20	10	0	5.54885	64887.3
4	10	0	0	2.6335	44518.3
5	10	10	20	4.46265	44377.2
6	0	10	0	2.89315	35791.2
7	0	0	0	0.18351	16783
8	20	20	0	6.7621	56713.4
9	0	20	20	4.2045	19848.6
10	0	20	10	3.7021	34152.4
11	20	20	20	5.61395	34592.6
12	20	0	0	4.7715	50099.6
13	20	0	20	5.10565	49023.4
14	0	20	0	3.36015	20371.4
15	20	10	10	3.4032	35153.9
16	0	0	20	2.8578	25383.1

[118] Through distribution analysis of the results for each condition as shown in Table 9 above, it was confirmed that the experimental design was D-optimal and that the experimental results were generally evenly distributed (FIG. 6).

[119] According to scatterplot matrix analysis, all correlation values were 0.75 or lower, indicating a weak correlation. The R values between the X variables (pea, wheat, and potato) were close to 0, indicating a very weak correlation, so there was no interaction. It was confirmed that there was a weak positive correlation between the Y variables (OD_{540nm}, and toxin concentration), between pea and the Y variable, and between wheat and the OD_{540nm} value (FIG.

that there were interactions between pea and potato, and between wheat and potato, but there were no interactions between the other factors (FIG. 11).

[124] [Table 10]

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Pea	1	1	19.476398	42.6410	0.0006*
Wheat	1	1	12.520622	27.4122	0.0019*
Potato	1	1	0.147585	0.3231	0.5904
Pea*Pea	1	1	0.695719	1.5232	0.2633
Pea*Wheat	1	1	0.789833	1.7292	0.2365
Wheat*Wheat	1	1	0.170263	0.3728	0.5639
Pea*Potato	1	1	2.003438	4.3863	0.0811
Wheat*Potato	1	1	2.272906	4.9762	0.0672
Potato*Potato	1	1	2.029966	4.4443	0.0796

[125] 2-7: Analysis of toxin expression level depending on type of plant peptone

[126] A fit model was established based on the results of ELISA. The model was established by the effect leverage method using least squares, and the X variables were analyzed using the response surface method. To select the optimal model, the R-squared value and RMSE value were considered together, and after checking effect summary and the p-value of effect test, terms that were not significant were deleted.

[127] As a result of checking the VIF value of the optimal established model, it was confirmed that the value did not exceed 10 and was at level 1, suggesting that there was no problem with the model. In residual analysis, it was confirmed that points were evenly distributed up and down

and all studentized residuals results were included in the 95% confidence interval (FIG. 12).

[128] As a result of identifying the factors that significantly affect the model through the effect test results, it was found that pea had an effect on the model (Table 11). A prediction formula for OD_{540nm} was obtained using this model (FIG. 13). As a result of examining the main effects and interaction profiles, it was confirmed that there were interactions between pea and potato, and between wheat and potato, but there were no interactions between the other factors (FIG. 14).

[129] [Table 11]

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Pea	1	1	1432636114	15.5150	0.0043*
Wheat	1	1	16083929.8	0.1742	0.6874
Potato	1	1	107423198	1.1634	0.3122
Pea*Pea	1	1	239492040	2.5936	0.1460
Wheat*Wheat	1	1	43348778.2	0.4695	0.5126
Pea*Potato	1	1	151433498	1.6400	0.2362
Wheat*Potato	1	1	94093681.4	1.0190	0.3423

[130] 2-8: Robust optimization

[131] Based on the prediction formula established for each response value, robust optimization conditions were identified using a profiler to determine the medium conditions that can obtain the appropriate OD_{540nm} and the maximum toxin concentration. In the actual culture process, for the OD_{540nm} value, a cell state is selected that can