

**Optimization and characterization studies of Poultry Waste Valorization for Peptone Production
Using a newly Egyptian *Bacillus subtilis* KEMET024 by Box-Behnken Design**

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Table S1: Nutritional and environmental factors with their low and high levels for the screening of peptone production using P6 isolate by Plackett-Burman design.

Factor	Name	Units	Minimum	Maximum
A	Feather meal	g/L	10.00	15.00
B	meat and bone	g/L	10.00	15.00
C	Starch	g/L	0.0000	20.00
D	Casein	g/L	0.0000	15.00
E	CaCO ₃	g/L	0.2000	0.5000
F	KH ₂ PO ₄	g/L	0.2000	0.5000
G	pH		6.00	8.00
H	temp.	°C	25.00	40.00

J	Inoculum size	%	3.00	7.00
K	Agitation	rpm	100.00	200.00
L	incubation time	days	24.00	48.00

I is a dummy factor by default using Design Expert 12 software.

Table S2: Plackett-Burman design matrix for peptone optimization by *B.subtilis* strain KEMET024

	Factor A: Feather meal (g/L)	Factor B: Meat and bone (g/L)	Factor C: Starch (g/L)	Factor D: Casei n (g/L)	Factor E: CaCO ₃ (g/L)	Factor F :KH ₂ P O ₄ (g/L)	Factor G: pH	Factor H: Temp (°C)	Factor J: Inocul um size %	Factor K: Agitati on rpm	Factor L: incuba tion days
1	+1	+1	-1	+1	+1	+1	-1	-1	-1	+1	-1
2	-1	-1	+1	-1	+1	+1	-1	+1	+1	+1	-1
3	-1	-1	-1	+1	-1	+1	+1	-1	+1	+1	+1
4	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
5	+1	+1	+1	-1	-1	-1	+1	-1	+1	+1	-1
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0
12	+1	+1	-1	-1	-1	+1	-1	+1	+1	-1	+1
13	+1	-1	+1	+1	-1	+1	+1	+1	-1	-1	-1
14	-1	+1	+1	+1	-1	-1	-1	+1	-1	+1	+1
15	+1	-1	-1	-1	+1	-1	+1	+1	-1	+1	+1
16	+1	-1	+1	+1	+1	-1	-1	-1	+1	-1	+1
17	-1	+1	+1	-1	+1	+1	+1	-1	-1	-1	+1
18	-1	+1	-1	+1	+1	-1	+1	+1	+1	-1	-1

Table S3: Nutritional factors with their low and high levels and their statistical screening matrix for the optimization of peptone production using *B.subtilis* strain KEMET024 by Box-Behnken design.

Nutritional factors with their low and high levels

Factor	Name	Level	Low Level	High Level
A	Meat and bone	10.00	5.00	10.00
B	starch	0.0000	0.0000	5.00
C	CaCO ₃	0.2500	0.0000	0.5000
Statistical screening of nutritional factors using Box-Behnken (PBD) design				
Run	Meat and bone	Starch	CaCO ₃	
1	0	0	0	
2	-1	0	+1	
3	0	0	0	
4	0	+1	-1	
5	0	0	0	
6	0	0	0	
7	0	+1	+1	
8	0	-1	-1	
9	0	-1	+1	
10	0	0	0	
11	+1	-1	0	
12	-1	+1	0	
13	-1	-1	0	
14	-1	0	-1	
15	+1	0	+1	
16	+1	0	-1	
17	+1	+1	0	

Figure S1: Comparative analysis of amino acid Profiles in commercial peptones and produced alternative from *B.subtilis* strain KEMET024

