

**Table S1** PB Design results

No.	Initial ratio of HSM to water (v : w)	Thickness of the substrate (cm)	Bacterial inoculum volume (v : w)	Relative numidity (%)	Temperature (°C)	Time (h)	Nattokinase activity (IU/g)
1	1 : 2.5	4	8	80	40	24	5789.7±134.2
2	1 : 1.5	4	12	70	40	24	5934.7±189.2
3	1 : 2.5	2	12	80	30	24	6208.5±256.7
4	1 : 1.5	4	8	80	40	16	5895.4±253.5
5	1 : 1.5	2	12	70	40	24	6373.6±96.3
6	1 : 1.5	2	8	80	30	24	6303.8±258.1
7	1 : 2.5	2	8	70	40	16	6215.2±213.6
8	1 : 2.5	4	8	70	30	24	6019.4±237.4
9	1 : 2.5	4	12	70	30	16	5914.6±268.9
10	1 : 1.5	4	12	80	30	16	6071.2±184.2
11	1 : 2.5	2	12	80	40	16	5931.4±209.8
12	1 : 1.5	2	8	70	30	16	6747.1±85.3

**Table S2** BBD results

No.	Initialratio of HSM to water(v : w)	Thickness of the substrate(cm)	Relative numidity(%)	Temperature( °C)	Nattokinase activity (IU/g)
1	1 : 1.5	2	75	35	6363.4±234.3
2	1 : 2.5	2	75	35	6438.2±87.9
3	1 : 1.5	4	75	35	6295.7±124.4
4	1 : 2.5	4	75	35	6236.5±133.7
5	1 : 2	3	70	30	6713.5±156.3
6	1 : 2	3	80	30	6746.7±216.5
7	1 : 2	3	70	40	6769.8±230.6
8	1 : 2	3	80	40	6772.7±156.9
9	1 : 1.5	3	75	30	6585.8±147.2
10	1 : 2.5	3	75	30	6663.7±184.9
11	1 : 1.5	3	75	40	6838.3±173.4
12	1 : 2.5	3	75	40	6508.4±166.2
13	1 : 2	2	70	35	6635.1±202.6
14	1 : 2	4	70	35	6646.9±152.7
15	1 : 2	2	80	35	6786.8±172.3
16	1 : 2	4	80	35	6543.9±98.4
17	1 : 1.5	3	70	35	6579.3±109.5
18	1 : 2.5	3	70	35	6381.3±127.4
19	1 : 1.5	3	80	35	6482.4±143.7
20	1 : 2.5	3	80	35	6518.2±138.1
21	1 : 2	2	75	30	6956.4±163.3
22	1 : 2	4	75	30	6602.4±177.8
23	1 : 2	2	75	40	6743.7±185.2
24	1 : 2	4	75	40	6734.9±107.4
25	1 : 2	3	75	35	7098.5±132.8
26	1 : 2	3	75	35	7003.2±146.2
27	1 : 2	3	75	35	7181.4±87.3
28	1 : 2	3	75	35	7076.3±118.9
29	1 : 2	3	75	35	7117.8±105.9