

## Supplementary Materials

**Table S1. Characteristics of the various proteases**

Enzyme	Main activity	Source	Optimum conditions	
			Temp. (°C)	pH
Alcalase® 2.4 L FG	Endo-protease	<i>Bacillus</i> sp.	50	7.8
Collupulin MG	Endo-protease	<i>Carica papaya</i>	50–70	5.0–7.5
Protamex	Broad-spectrum endo-protease	<i>Bacillus</i> sp.	35–60	5.5–7.5
Flavourzyme® 500 MG	Protease (endo & exo)	<i>Aspergillus oryzae</i>	50	5.0–7.0
Prozyme 2000P	Exo-peptidase	<i>A. oryzae</i>	50–60	6.0–9.0

**Table S2. Combination of experimental runs based on RSM: Mixing ratio of enzymes**

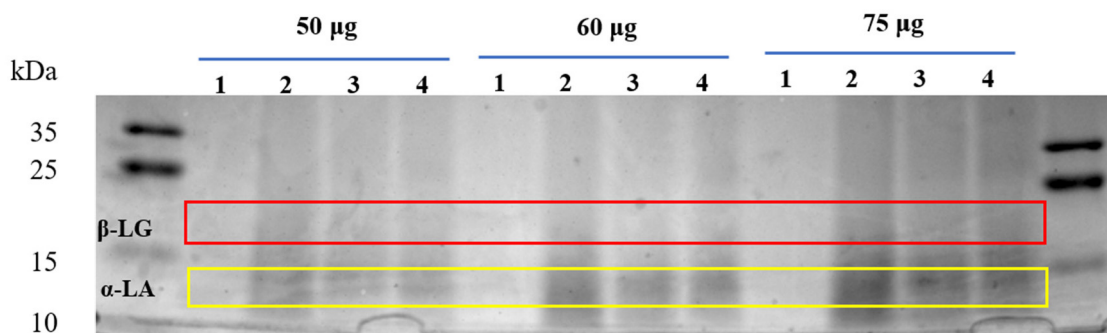
RUN	Substrate (%)	Alcalase (%)	Prozyme (%)
1	10	0.5	0.5
2	10	1	0
3	10	0.5	0.5
4	10	0.75	0.25
5	10	0.75	0.25
6	10	1	0
7	10	0.5	0.5
8	10	0	1
9	10	0.25	0.75
10	10	0	1
11	10	1	0
12	10	0.25	0.75
13	10	0.25	0.75
14	10	0	1
15	10	0.75	0.25

RSM, response surface methodology.

**Table S3.** Combination of experimental runs based on RSM: Reaction conditions (enzyme addition amount and hydrolysis time) for mixed enzymes

RUN	Substrate (%)	Enzyme (%)	Time (h)
1	10	2	6
2	10	2	6
3	10	1	6
4	10	1.5	4
5	10	1	2
6	10	1	10
7	10	1.5	8
8	10	1	2
9	10	1.5	8
10	10	0.5	6
11	10	2	6
12	10	1	6
13	10	1	6
14	10	1	6
15	10	1	6
16	10	1.5	4
17	10	0.5	6
18	10	1	6
19	10	0.75	8
20	10	0.75	4
21	10	0.5	6
22	10	1	6
23	10	1	10
24	10	1	6
25	10	0.75	8
26	10	1	6
27	10	0.75	4
28	10	1	2
29	10	1.5	4
30	10	0.75	4
31	10	1	6
32	10	1	6
33	10	1	6
34	10	1	6
35	10	1.5	8
36	10	1	6
37	10	1	10
38	10	1	6
39	10	0.75	8

RSM, response surface methodology.



**Fig. S1.** Gel electrophoresis of whey protein hydrolysis for the analysis of  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin. Lane 1: hydrolysate prepared by treatment with prozyme and alcalase, Lane 2: hydrolysate prepared by treatment with prozyme and collupulin, Lane 3: hydrolysate prepared by treatment with prozyme and protamex, Lane 4: hydrolysate prepared by treatment with prozyme and flavourzyme. Protein concentration (50, 60, and 70  $\mu$ g) of samples loaded on gel.