

1 **Effects of the thermal denaturation degree of a whey protein isolate on the strength of acid**
2 **milk gels due to the dissociation of κ -casein**

3

4 Daiki Oka^{1*}, Wataru Ono², Shojiro Tamaki³, Tomohiro Noguchi², and Katsumi Takano¹

5

6

7

8 **SUPPLEMENTARY FILE**

9

10 **Materials & Methods**

11 *Quantitation of κ -Casein*

12 Upon heating of WPI-containing skimmed milk, κ -casein dissociated from casein micelles
13 (Mohomud et al., 2017), and the extent of this dissociation was quantified by reverse-phase high-
14 performance liquid chromatography using the method of Bonfatti et al. (2008). The milk sample
15 was subjected to ultracentrifugation (33,000 g, 30 min) (himac CP80WX, HITACHI), and the
16 supernatant was dissolved in 0.1 M phosphate buffer (pH 6.8) to a protein content of 2 mg/mL. A
17 850- μ L aliquot of this solution was then treated with 50 μ L of 0.5 M dithiothreitol containing 50
18 mM ethylenediaminetetraacetate, heated for 10 min, supplemented with 100 μ L of 0.5 M
19 iodoacetamide to modify the SH groups produced, and passed through a 0.45- μ m filter. A 50- μ L
20 aliquot of the prepared sample was loaded on a ZORBAX 300SB-C8 column (Agilent) and
21 eluted at 0.5 mL/min using a gradient of two solvents. Solvent A consisted of 0.1 wt%
22 trifluoroacetic acid (TFA) in water, and Solvent B was 0.1 wt% TFA in acetonitrile. Separations
23 were performed with the following program: 0–8 min linear gradient from 30% B to 37% B; 8–
24 12 min isocratic elution 37% B; 12–18 min linear gradient from 37% B to 40% B; 18–23 min
25 isocratic elution 40% B; 23–27 min linear gradient from 40% B to 80% B; 27–31 min isocratic
26 elution 80% B; and 31–34 min linear gradient from 80% B to 30% B, followed by a 6-min
27 isocratic elution under the initial conditions. Therefore, the total duration of a single run,
28 including column re-equilibration, was 40 min. The detection was performed at a wavelength of
29 214 nm. Peak areas were determined by fitting to a calibration curve constructed using a standard
30 solution, and the ratio of κ -casein content in the whey fraction to that in milk was defined as the
31 extent of κ -casein dissociation (%).

32

33 **Figure 1:**

34

35

36

37

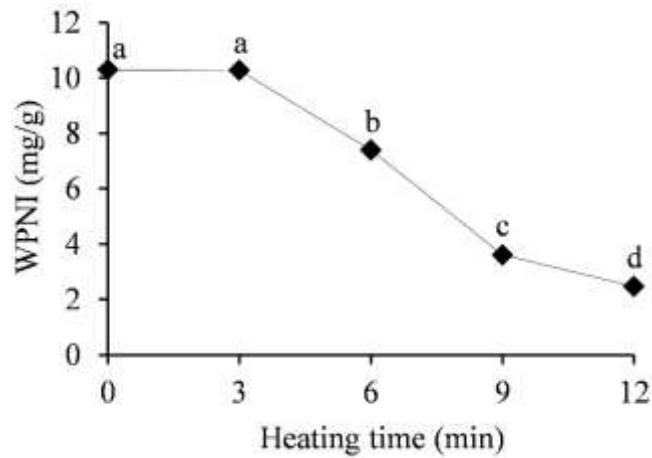
38

39

40

41

42



43 Effect of heating time (0, 3, 6, 9, or 12 min at 80 °C) of whey protein isolate (WPI) on whey
44 protein nitrogen index (WPNI). WP was diluted 40-fold with deionized water and heated at 80
45 °C for 3, 6, 9, or 12 min. Error bars represent the standard deviation of three measurements. a-f
46 Means within a row with different superscripts differ significantly (Tukey–Kramer test, $P <$
47 0.01).

48

49 **Figure 2:**

50

51

52

53

54

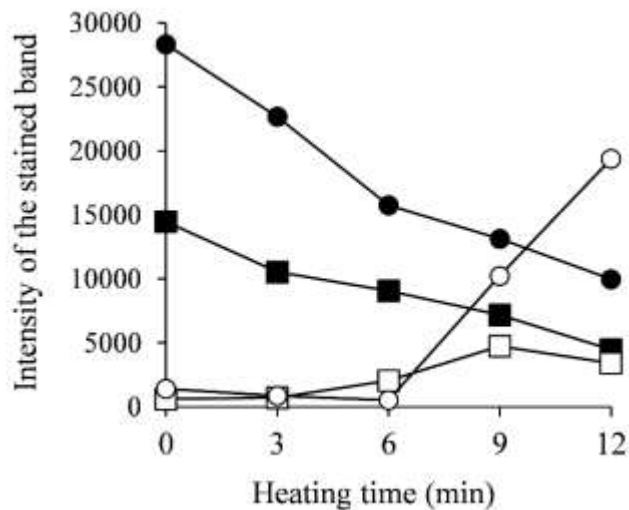
55

56

57

58

59



60 Change in the staining band intensity of non-reducing SDS-PAGE. The figure shows the bands
61 of β -lactoglobulin (closed circle), α -lactalbumin (closed square), Aggregates I (open square), and
62 Aggregates II (open circle).