Supplementary materials

Preparation and optimization of ginger-juice milk curd

1 Materials and methods

1.1 Preparation of ginger-juice milk curd

Seed-ginger rhizomes (Zingiber, produced in Shandong Province, China and bought from a food market near Zhejiang University of Science and Technology in Hangzhou, China) were cleaned, peeled, and cut into pieces. Then, they were put into a juicer (SJ09-200; Supor) and the ginger juice obtained was filtered using four layers of gauze to remove the precipitates and residue.

Appropriate amounts of milk powder (10%-18%, 0.10-0.18 g/mL), sucrose (10%, 0.10 g/mL) and calcium chloride (0.005%-0.025%, 0.05-0.25 g/L) were weighed then mixed with the proper amount of distilled water. Next, the pH of milk was adjusted to the appropriate value (4.5-6.5) with citric acid.

The milk was heated or cooled to different temperatures (50-90°C) after pasteurization at 95°C for 5 min. Then, appropriate amounts of ginger juice (3%-7%, volume fraction) were poured into the milk, mixed evenly in a short time and allowed to stand for a few minutes before coagulation.

1.2 Analysis of hardness

The hardness of ginger-juice milk curd was determined using a texture analyzer TA-XT Plus C (Stable Micro Systems, UK). A P30 probe (30 mm in diameter) with a round flat surface was used. Before the probe touched the sample surface, its speed was 0.5 mm/s. The speed was 15 mm/s during measurement and the probe retracted at a speed of 10 mm/s. The compression distance was 28 mm and the pressure was 10 g. Each sample was measured in triplicate.

1.3 Analysis of water holding capacity

About 10 g of evenly stirred curd was weighed into a tube, and centrifuged at 6000 r/min for 15 min. Then we removed the supernatant and weighed the precipitate. The WHC was evaluated using following equation:

$$WHC=(w_1-w_2)/w_1\times 100\%,$$

where w_1 is the weight of ginger-juice milk curd before centrifugation and w_2 is the weight after centrifugation. Each sample was tested in triplicate.

1.4 Sensory analysis

Quantitative descriptive analysis of the ginger-juice milk curd was carried out by a panel with ten students (mean age of 24±3) from the University who were trained to know how to score the samples on their appearance, coagulation, aroma and taste. Descriptive analysis attributes for the ginger-juice milk curd are shown in Table 1.

Table S1 Descriptive analysis for the ginger-juice milk curd

Attribute	Definition	Grade
Appearance	Ivory or light yellow; Uniform glossy color; Smooth and surface;	20-25
	Ivory or light yellow; Uneven and poor color; Unsmooth surface;	10-20
	Dark color or other colors; Unsmooth surface;	0-10
Coagulation state	Complete curd; No or few whey; No liquidity;	20-25
	Relatively complete curd; More whey; Liquidity;	10-20
	Incomplete curd; A large amount of whey; Strong liquidity;	0-10
Aroma	Strong aroma of ginger and milk; No other odor;	20-25
	Light aroma of ginger and milk;	10-20
	Odor;	0-10
Taste	Creamy taste; Moderate sweet and spicy;	20-25
	Creamy taste; Sweet or spicy;	10-20
	Not creamy taste; Too sweet or too spicy;	0-10

2 Results

To better understand the changes of various properties in the curding process with ginger juice, the preparation process was optimized. Firstly, the effects of the addition of ginger juice, temperature of milk, pH of milk, and addition of calcium chloride and addition of milk powder on the WHC and hardness of ginger-juice milk curd were investigated (Fig. S1).

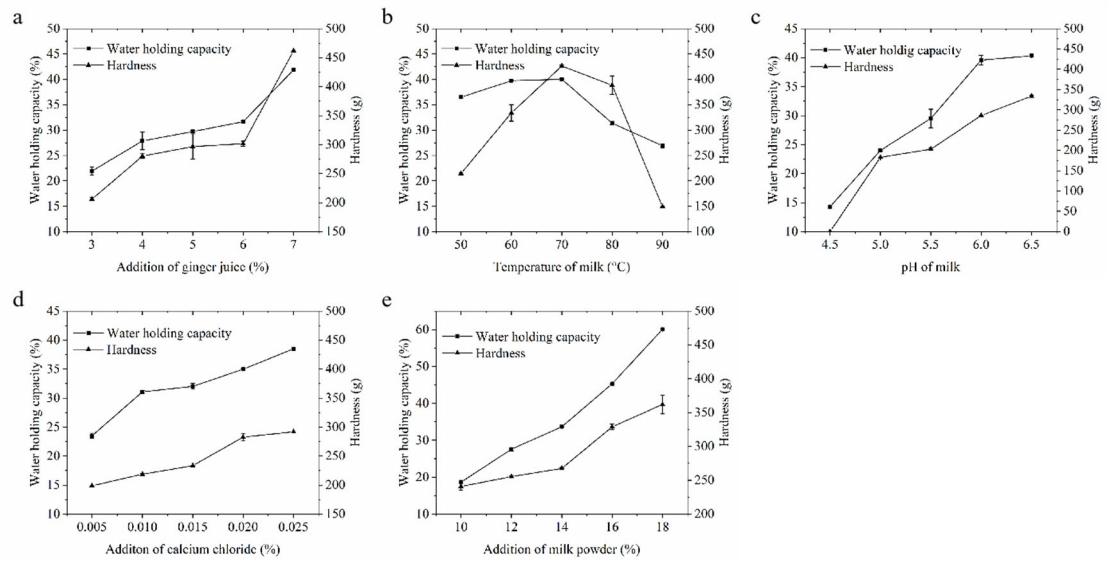

Fig. S1 Effects of the addition of ginger juice (a), temperature of milk (b), pH of milk (c), the addition of calcium chloride (d) and milk powder (e) on water holding capacity and hardness.

The WHC and hardness of ginger-juice curd increased with the enhancement of the amount of ginger juice added (Fig. S1a), indicating that the addition of ginger juice had a positive effect on coagulation of milk. The WHC and hardness values changed little at the concentrations of 4%, 5% and 6%, but changed obviously at 3% and 7%. However, the more ginger juice added, the spicier it tasted: 3% of ginger juice flavor was insufficient, but 7% was too spicy to accept. Therefore, the concentration of 4 to 5% was further optimized. The optimum temperature for both WHC and hardness was 70°C (Fig. S1b), while cotton-shaped precipitates formed when the temperature was higher than 70°C. Therefore, 50 to 70°C was chosen for further optimization. The factors of pH (Fig. S1c), the addition of calcium chloride (Fig. S1d) and milk powder (Fig. S1e) on the WHC and hardness showed positive effect on coagulation of milk. The ginger-juice milk curd coagulated incompletely when the pH was less than 5.5, and so pH 5.5 to 6.5 was selected for further study. The curd was not good and whey precipitates were formed when the concentration of calcium chloride was less than 0.015%, while the taste was rough and not delicate when it was over 0.025%. When the concentration of milk powder was over 14%, the milk was hard to mix well and became viscous and thick, which might be not good for preparation of ginger-juice milk curd. Therefore, 10% to 14% was chosen for further optimization.

After studying five single factors, Plackett-Burman analysis was carried out to select the significant factors according to the WHC (Table S2).

Table S2 Experimental scheme of the five single factors on water holding capacity

RunOrder	Addition of ginger juice (%)	Temperature of milk (°C)	pH of milk	Addition of calcium chloride	Addition of milk powder (%)	Water holding capacity (%)
1	6	70	5.5	0.025	10	9.41±0.81
2	4	50	6.5	0.025	14	40.66±0.29
3	4	70	5.5	0.015	10	4.96±0.49
4	4	50	5.5	0.015	10	3.21±1.09
5	6	50	6.5	0.015	10	41.45±1.54
6	6	50	6.5	0.025	10	43.82±0.32
7	6	70	5.5	0.025	14	9.70±0.09
8	4	70	6.5	0.025	10	40.18±0.61
9	4	70	6.5	0.015	14	35.07±0.28
10	6	70	6.5	0.015	14	40.23±0.29
11	6	50	5.5	0.015	14	19.14±1.75
12	4	50	5.5	0.025	14	8.61±1.28

Variance analysis (Table S3) showed that the pH of milk and the addition of ginger juice were significant factors ($P<0.05$). The temperature was also an important factor. The addition of calcium chloride and milk powder were not significant factors. Based on the results of single factor analysis, 0.02% and 12% were selected, respectively.

Table S3 Variance analysis of the five single factors

Source	Freedom	Adj SS	Adj MS	F value	P value
Model	5	3014.99	603.00	48.70	0.000
Linear	5	3014.99	603.00	48.70	0.000
Addition of ginger juice	1	80.39	80.39	6.49	0.044
Temperature of milk	1	25.06	25.06	2.02	0.205
pH of milk	1	2894.79	2894.79	233.79	0.001
Addition of calcium chloride	1	5.77	5.77	0.47	0.520
Addition of milk powder	1	8.98	8.98	0.73	0.427
Error	6	74.29	12.38		
Amount to	11	3089.28			

These three significant factors were further optimized by orthogonal design according to sensory analysis (Table S4).

Table S4 Experimental scheme of three factors on sensory analysis

Run	Factor 1 A: Addition of ginger juice %	Factor 2 B: pH of milk	Factor 3 C: Temperature °C	Response 1 Sensory analysis
1	4.00	6.00	50.00	73±2.83
2	5.00	6.50	50.00	84±3.31
3	5.00	6.00	60.00	96±3.06
4	5.00	6.50	70.00	85±1.41
5	5.00	6.00	60.00	95±2.78
6	5.00	5.50	50.00	54±2.71
7	5.00	5.50	70.00	57±1.70
8	6.00	5.50	60.00	36±3.11
9	4.00	6.50	60.00	87±3.19
10	6.00	6.00	70.00	70±2.69
11	5.00	6.00	60.00	95±1.94
12	6.00	6.00	50.00	69±1.70
13	5.00	6.00	60.00	95±2.87
14	6.00	6.50	60.00	93±2.24
15	5.00	6.00	60.00	94±2.33
16	4.00	5.50	60.00	48±3.30
17	4.00	6.00	70.00	84±2.52

The optimum process conditions were that the addition of ginger juice was 4.97%, the pH of milk was 6.29 and the temperature was 61.06°C (Fig. S2).

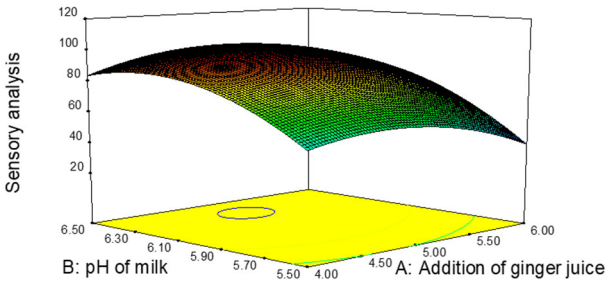

Fig. S2 3D-surface diagram of orthogonal experiment.