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Research Article

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Dynamic changes in physiochemical, structural, and flavor characteristics of ginger-juice milk curd

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Abstract: Dynamic changes in the physiochemical, structural, and flavor characteristics of ginger-juice milk curd were explored by texture analysis, scanning electron microscopy, rheometry, electronic tongue, and gas chromatography-mass spectrometry (GC-MS). Protein electrophoresis showed that ginger juice could hydrolyze α_s-, β-, and κ-casein. Curd formation was initiated at 90 s, marked by significant changes in intensity detected via intrinsic fluorescence. The contents of soluble protein and calcium decreased rapidly during coagulation, while the caseinolytic activity, storage moduli, loss moduli, hardness, adhesiveness, and water-holding capacity increased, resulting in a denser gel structure with smaller pores and fewer cavitations as observed by scanning electron microscopy. Electronic tongue analysis indicated that milk could neutralize the astringency and saltiness of ginger juice, rendering the taste of ginger-juice milk curd more akin to that of milk. Approximately 70 volatile components were detected in ginger-juice milk curd. α-Zingiberene, α-curcumene, β-sesquiphellandrene, and β-bisabolene were the predominant volatile flavor compounds, exhibiting an initial decrease in content followed by stability after 90 s. Decanoic acid, γ-elemene, and caryophyllene were identified as unique volatile compounds after mixing of milk and ginger juice. Understanding the dynamic changes in these characteristics during coagulation holds significant importance for the production of ginger-juice milk curd.

Key words: Ginger; Milk coagulation; Characterization; Volatile compounds

1 Introduction

Ginger-juice milk curd, locally called "Jiang Zhuang Nai," can be obtained by adding ginger juice to milk at an appropriate temperature and placing it in a static state at room temperature (Zhong and Chen, 2017). Originating from Guangdong and Fujian Provinces in China, this dessert is cherished for its mellow taste, unique aroma, and health benefits. With advancements in food-processing techniques, there is a growing trend toward producing healthier foods using natural bioactive compounds (Castro-Muñoz et al., 2019), such as gingerol (Garza-Cadena et al., 2023), natural sweetener (Castro-Muñoz et al., 2022a), capsaicin (Castro-Muñoz et al., 2022b), anthocyanin (Hernández-Pinto et al., 2024), and carminic acid (Ferreyra-Suarez et al., 2024). Such foods are gaining popularity because of the increasing health consciousness of consumers and the perceived nutritional benefits and functional properties of such compounds.

Ginger is widely used as a seasoning, traditional medicine, and natural food additive and provides a unique spicy and warm taste sensation (Indiarto et al., 2021). Its aroma and taste are attributed to sesquiterpenes, oxygenated monoterpenes, and sesquiterpenoids (Nagendra Chari et al., 2013). It is rich in lipid, protein, vitamin, carotene, and other nutrients such as calcium, iron, and phosphorus (Haji Ghafarloo et al., 2020). The primary functional active components of ginger include ginger protease, gingerol, shogaol, zingiberene, flavone, oleoresin, zingberol, and zingniberone (Garza-Cadena et al., 2023). The extracted compounds have functional and pharmacological properties such as antioxidant, antibacterial, anticarcinogenic, antilipidemic, antitumor, antimutagenic, blood sugar-lowering, stomach-warming, blood circulation-promoting, and immune system-enhancing effects, making ginger a highly valuable functional food ingredient (Mancini

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et al., 2017; El-Ashmawy et al., 2018; Li et al., 2019; AlTamimi et al., 2020; Garza-Cadena et al., 2023).

Most studies on ginger-juice milk curd have focused mainly on the milk-clotting factors and optimization of manufacturing conditions. Zhong and Chen (2017) found that the temperature and pH of milk and the addition of ginger juice and calcium chloride all affected its taste and quality. Zeng et al. (2007) observed that only crude ginger protease extract could coagulate milk, while ginger essential oil or ginger oleoresin could not. Liu (2012) confirmed that ginger protease was the main factor in ginger-juice milk curd formation, potentially offering a cheaper alternative to rennet (Hashim et al., 2011). κ-Casein is found in the shell of casein micelles. Ginger protease can hydrolyze κ-casein at Ala90-Glu91 and His102-Leu103, resulting in the exposure of α_{s1}- and β-casein. The Ca^{2+} then interacts with casein to form a three-dimensional (3D) network structure, causing ginger-juice milk curd to transition to a gel state (Huang et al., 2011). Jiang and Li (1998) found that other substances in ginger such as gingerol, shogaol, and zingerone can also help to coagulate milk. However, there have been few studies on the texture, microstructure, and flavor compounds in the process of coagulation. Therefore, in this study, we investigated the dynamic changes in physiochemical, microstructural, and flavor characteristics during coagulation of ginger-juice milk curd, contributing to a better understanding of its coagulation mechanism and offering valuable insights for ginger utilization.

2 Materials and methods

2.1 Preparation of ginger-juice milk curd

Ginger-juice milk curd was prepared according to Yang et al. (2012). To better understand the changes in various properties during coagulation, the preparation procedure was optimized (supplementary materials and methods). Milk powder (12%, 0.12 g/mL), sucrose (10%, 0.10 g/mL), and calcium chloride (0.02%, 0.2 g/L) were weighed and mixed with distilled water. The pH of the milk was adjusted to 6.29 with citric acid. The milk was cooled to 61 °C after pasteurization at 95 °C for 5 min. Then, ginger juice (5%, volume fraction) was poured into the milk, mixed evenly for a short time, and standed for a few minutes before coagulation.

2.2 Analysis of hardness

The hardness of ginger-juice milk curd was determined using the TA-XT Plus C texture analyzer (Stable Micro Systems, UK) following the methods of Li et al. (2021). A P30 probe (30 mm in diameter) with a round flat surface was used. Before the probe touched a sample's 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 depth of compression was 28 mm and the pressure was 0.098 N. Each sample was measured in triplicate.

2.3 Analysis of water-holding capacity

The water-holding capacity (WHC) of each ginger-juice milk curd sample was measured according to the methods reported by Huang et al. (2021). Approximately 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(C_{WH}) was evaluated using the following equation:

$$C_{WH}=(w_1-w_2)/w_1\times 100\%,$$

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.

2.4 Determination of total titratable acidity

Total titratable acidity (TTA, A) was measured in 5 g of ginger-juice milk curd blended with 40 mL distilled water. The sample was titrated with 0.1 mmol/L NaOH, and phenolphthalein was used as the indicator. Three replicates were averaged for each sample.

$$A=10\times cV/m,$$

where c is the actual molar concentration of NaOH, V is the volume of NaOH used, and m is the weight of ginger-juice milk curd.

2.5 Determination of the calcium content

Ginger-juice milk curd samples with different clotting times (0, 30, 60, 90, 180, 300, 420, and 600 s) were filtered with filter paper after stirring evenly. A volume of 5 mL of filtrate was mixed with 45 mL of distilled water and the pH was adjusted to 13 with 20% (0.2 g/mL) NaOH. Calred was used as the indicator and each sample was titrated with 0.01 mol/L ethylene diamine tetraacetic acid (EDTA) standard solution.

2.6 Determination of the soluble protein content

The content of soluble protein was measured according to the Bradford method (Field and Field, 2010). Ginger-juice milk curd samples with different clotting times (0, 30, 60, 90, 180, 300, 420, and 600 s) were diluted 500 times with distilled water and mixed with 5 mL of Coomassie brilliant blue solution, and the absorbance was monitored at 595 nm.

2.7 Protein electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the procedure of Atasever et al. (2013). Crude bovine casein was prepared according to Sharma et al. (2021). Milk powder (1 g) was dissolved in distilled water (10 mL) and the pH was adjusted to 4.6 with 1 mol/L HCl. Then the sample was centrifuged at 3000 r/min for 15 min. The supernatant was discarded and the precipitate was washed three times with water and once with 95% ethanol, and then dried at 55 °C to obtain crude bovine casein.

2.8 Determination of the caseinolytic activity of ginger protease

The caseinolytic activity of ginger protease in ginger juice and in ginger-juice milk curd with different clotting times (0, 30, 60, 90, 180, 300, 420, and 600 s) was determined following the method of Huang et al. (2011). Samples were diluted 500 times with distilled water. The diluted solution (2 mL) was mixed with 2 mL of 1% (0.01 g/mL) casein and reacted at 40 °C for 4 min. Then 4 mL of 0.4 mol/L trichloroacetic acid was added to terminate the reaction. The blank was prepared by adding trichloroacetic acid without a reaction. After filtering the reaction solution, the absorbance of permeate was monitored at 275 nm. One unit of caseinolytic activity was defined as the amount of the enzyme that generated 1 μg of tyrosine per min at 40 °C.

2.9 Observation of microstructure

Samples of ginger-juice milk curd with different clotting times (0, 30, 60, 90, 180, 300, 420, and 600 s) were examined by scanning electron microscopy (SEM) according to the method of Fang and Guo (2019). Samples were fixed in 2.5% (0.025 g/mL) glutaraldehyde at 4 °C for 4 h, washed three times with distilled water, and dehydrated using a series of increasing ethanol concentrations (30%, 50%, 70%, 90%, and 100%, volume fraction). Finally, the dehydrated samples were frozen at −80 °C overnight and freeze-dried for 2 d.

2.10 Analysis of rheological properties

A Discovery Series Hybrid Rheometer DHR-3 (TA Instruments, New Castle, USA) was used to characterize the rheological properties of ginger-juice milk curd as described by Pachekrepapol et al. (2020) with slight modifications. The rheometer was equipped with a plate and cone geometry (40 mm in diameter) with a 1-mm gap and operated at 25 °C. Ginger-juice milk curd homogeneously stirred was pre-sheared for 30 s at a shear rate of 300 s^{-1}, followed by equilibration for 300 s for structural recovery. A strain sweep of ginger-juice milk curd with different clotting times (1.5, 3, 5, 7, and 10 min) from 0.01% to 100% at a constant frequency of 1 Hz was carried out to determine the linear viscoelastic region. The storage modulus (G') of each sample was in the linear viscoelastic region between 0.01% and 0.50%. Therefore 0.50% was selected as the constant strain for the frequency sweep test from 0.1 to 10.0 Hz.

2.11 Analysis of intrinsic fluorescence

Ginger-juice milk curd was diluted with 1 mol/L phosphate buffer (pH 7.0) to 200 μg/mL and detected in an enzyme-linked immunosorbent assay (ELISA) reader with an emission wavelength of 330–480 nm and a slit width of 5 nm.

2.12 Electronic tongue analysis

Electronic tongue analysis was performed according to the method of Zhang et al. (2019) with some modifications. Ginger juice (5 mL), milk (5 mL), and ginger-juice milk curd (5 g) were each mixed with distilled water and centrifuged. Then the supernatant was filtered and obtained for electronic tongue analysis using a Taste Sensing System E-tongue TS-5000Z (Intelligent Sensor Technology, Inc., Japan) equipped with five chemical sensors for sourness, bitterness, astringency, saltiness, and sweetness. Each sample was measured in triplicate.

2.13 Analysis of volatile compounds

The volatile compounds in ginger-juice milk curd were determined by headspace solid-phase microextraction (HS-SPME) according to the method of Tian et al. (2021) with slight modifications. Ginger juice, milk, and ginger-juice milk curd (3 g) with different clotting times (0, 30, 60, 90, and 600 s) were each mixed with 25% (0.25 g/mL) sodium dihydrogen phosphate (3 mL) in different headspace vials. The samples were stirred by magnetic force for 30 min at 50 °C. An aged fiber was inserted into sample vials and the volatile substances were extracted for half an hour at 50 °C, with magnetic stirring. Then, the fiber was removed and quickly inserted into the gas chromatograph-mass spectrometer 5975C (Agilent Technologies, USA) inlet. The temperature program and the carrier gas were the same as those used in the method of Delgado et al. (2010).

3 Results and discussion

3.1 Physiochemical characterization

We found that curd could not form before 60 s, but started to form after 90 s and was completely coagulated in 10 min. According to the previous optimized conditions (supplementary materials and methods), the ginger-juice milk curd exhibited the following characteristics: hardness of 305.416 gf (1 gf=0.009 81 N), adhesiveness of 94830 mPa·s, gumminess of 226.937, cohesiveness of 0.743, and TTA of 21.38 °T (1 °T= 0.009 g/mL).

We investigated the changes in physicochemical properties during the 10 min-coagulation process (Fig. 1). The hardness increased rapidly from 90 s to 5 min, followed by a tendency to plateau, indicating that the curd is basically formed within 5 min (Fig. 1a). The adhesiveness (Fig. 1b) and WHC (Fig. 1c) also showed an upward trend during coagulation, albeit with WHC showing a comparatively slower enhancement between 3 and 7 min. Although the network structure of curd becomes more compact during coagulation, perhaps there may still be some interstitial spaces leading to water loss. WHC increased from 25.17% to 30.79% after 7 min.

Zeng et al. (2007) found that ginger protease plays an important role in coagulation. Therefore, the changes of caseinolytic activity of ginger protease and the content of soluble protein during coagulation were investigated (Fig. 1d). The content of soluble protein was 7.70 mg/mL and the caseinolytic activity of ginger juice was 17.48 U, while those of milk were 16.73 mg/mL and 0 U, respectively. When ginger juice was mixed with milk, namely at 0 s of clotting time, the content of soluble protein was 24.01 mg/mL and the caseinolytic activity was 10.08 U. Within the first minute, the content of protein decreased rapidly, while the caseinolytic activity increased rapidly, peaking at 13.11 U. At the beginning, this rise in casein hydrolysis activity facilitated protein hydrolysis, leading to a rapid decline in soluble protein content. This decline plateaued at around 10.60 mg/mL after 90 s, coinciding with the basic formation of milk curd. Subsequently, caseinolytic activity gradually decreased from 60 s to 10 min due to inactivation by high temperature, ultimately leading to complete loss of activity over time. In general, the trend of caseinolytic activity was consistent with that of the protein content. Owing to the instability of ginger protease, the curd must be prepared fresh. Furthermore, the storage stability of the curd is poor, with whey separation occurring after storing at

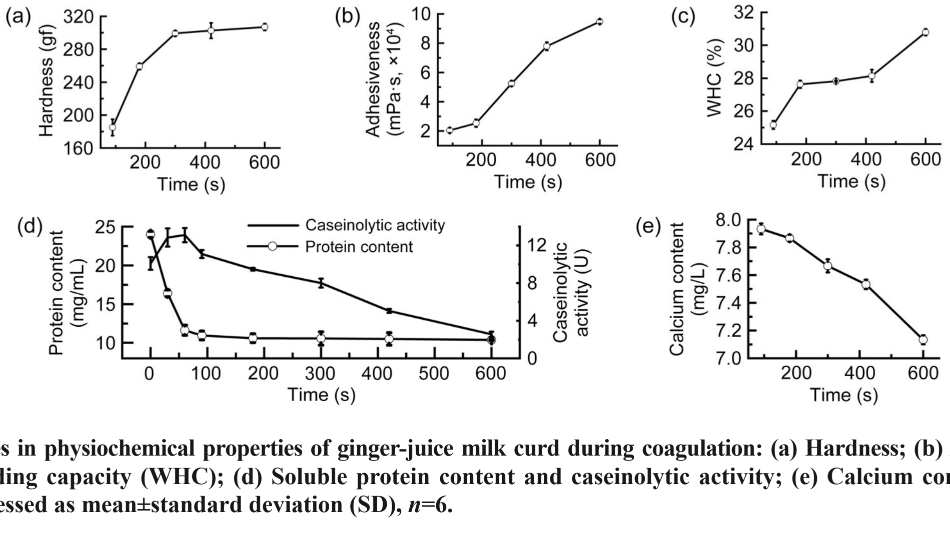

Fig. 1 Changes in physiochemical properties of ginger-juice milk curd during coagulation: (a) Hardness; (b) Adhesiveness; (c) Water-holding capacity (WHC); (d) Soluble protein content and caseinolytic activity; (e) Calcium content of whey. Data are expressed as mean±standard deviation (SD), *n*=6.

4 °C for 2 d, resulting in a loss of freshness. Therefore, improving the stability of ginger protease and extending the shelf life of ginger-juice milk curd are crucial areas for future research.

Ginger protease can hydrolyze κ-casein, which acts as the shell of the casein micelle. This hydrolysis leads to the exposure of α_s- and β-casein. Then the Ca^{2+} interacts with the exposed casein proteins to form a 3D network structure, causing ginger-juice milk curd to exhibit a gel state. As the clotting time went by, more α_s- and β-casein were exposed, allowing them to bind with additional Ca^{2+}. Therefore, the content of calcium in the whey decreases gradually throughout the coagulation process (Fig. 1e).

SDS-PAGE was carried out to verify the changes of the protein in ginger-juice milk curd during coagulation (Fig. 2). There were two obvious bands in lane a: one around 33 kDa and the other between 10 and 17 kDa. These bands correspond to ginger proteinase, consistent with the results reported by Zhong and Chen (2017). Milk protein is composed of approximately 80% casein and 20% lactoal-bumin. Casein can be divided into α_s-, β-, and κ-casein, accounting for approximately 40%, 32%, and 8% of the total milk protein, respectively. Lactoal-bumin consists mainly of β-lactoglobulin and α-lactalbumin (Sharma et al., 2021). A total of 10 and 3 μL of crude bovine casein were loaded into lane b and lane c, respectively. In lane b, there were three bands located at 25–33, 17–25, and 10–17 kDa, which may have corresponded to casein, impurified β-lactoglobulin, and α-lactalbumin, respectively. The unrecognizable bands between 25 and 33 kDa in lane b were clearly shown by reducing the volume of sample, revealing three bands corresponding to α_s-, β-, and κ-casein from top to bottom (Sharma et al., 2021). The band of κ-casein was almost invisible in ginger-juice milk curd at different times because of its relatively low content and rapid degradation by ginger protease. In addition, as the clotting time increased, the concentrations of α_s- and β-casein decreased (Fig. S1), suggesting hydrolysis by ginger protease, destabilizing casein micelles, and leading to gel formation. However, these results contrast with those reported by Raynal-Ljutovac et al. (2007). They found that ginger protease hydrolyzed almost no α_{s1}-, α_{s2}-, or β-casein. It acted mainly on the Thr121–Ile122 peptide bond of κ-casein, producing the N-terminal of the accessory κ-casein called κ-CN (f1–121) and the C-terminal casein peptide, which caused casein to lose stability and coagulate into gel.

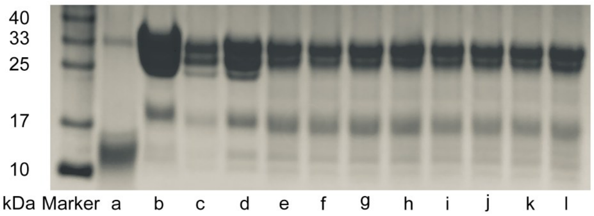

Fig. 2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins during coagulation. (a) Ginger juice; (b) 10 μL of crude bovine casein; (c) 3 μL of crude bovine casein; (d) milk; (e–l) ginger-juice milk curd loaded successively at different clotting times: 0 (e), 30 (f), 60 (g), 90 (h), 180 (i), 300 (j), 420 (k), and 600 s (l).

3.2 Microstructure

The microstructure of curd at different clotting times was examined by SEM at 1000× or 5000× magnification, revealing gradual agglutination of the gel network (Fig. 3). The gel network could not form within 60 s (Figs. 3a and 3b) and first appeared at 90 s (Fig. 3c), which suggested that by then the ginger-juice milk curd no longer presented as liquid but had basically coagulated. The gap of the gel network was slightly larger and the structure was loose at 3 min (Fig. 3d). Coagulation was complete at 5 min (Fig. 3e). As the clotting time went by, the microstructure of samples showed smaller pores, fewer cavitations, and a more compact gel structure (Figs. 3f–3h).

3.3 Rheological properties

The rheological properties of ginger-juice milk curd with different clotting times are shown in Fig. 4. The storage moduli (G') represent the elastic characteristics of materials, while the loss moduli (G'') represent the viscous properties. Both G' and G'' increased as the frequency rose, indicating that the structure of ginger-juice milk curd became denser and that its texture was finer, which is consistent with the results of SEM. In addition, G' was always greater than G'', indicating that ginger curds exhibit a fluid characteristic of elasticity greater than viscosity at different clotting times (Olojede et al., 2020). The results showed that the coagulating ingredient in ginger juice could hydrolyze the high molecular weight proteins in milk and obtain smaller molecular weight proteins, which was conducive to the formation of a more compact gel structure.

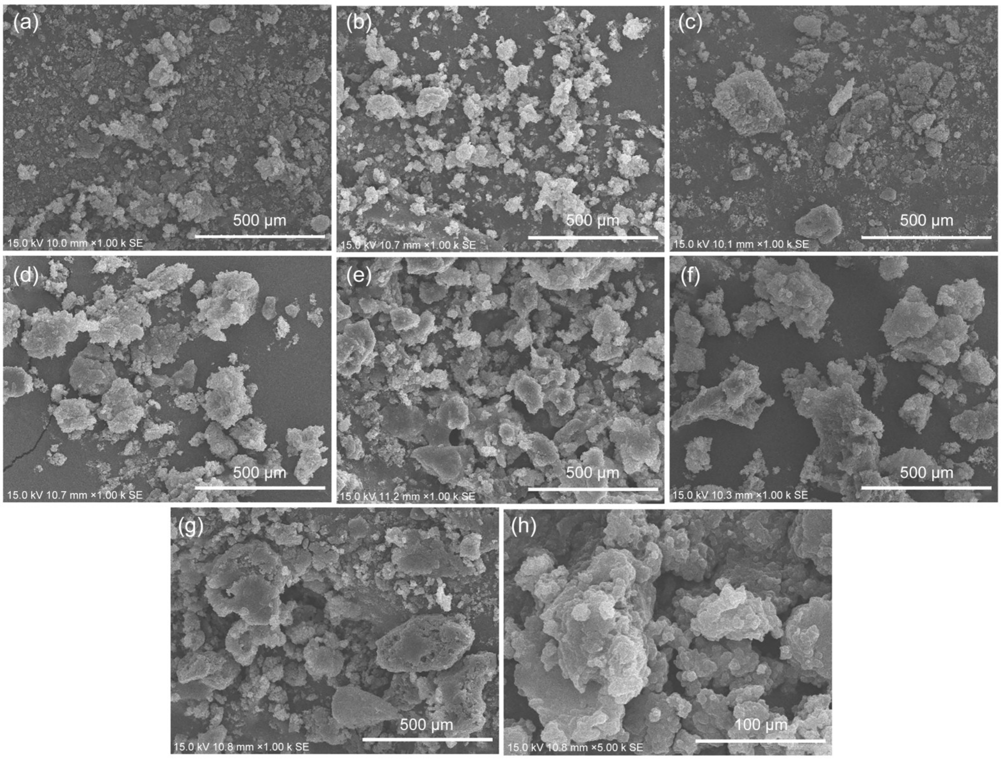

Fig. 3 Microstructure of ginger-juice milk curd at different clotting times analyzed by scanning electron microscopy. The samples at 30 (a), 60 (b), 90 (c), 180 (d), 300 (e), 420 (f), and 600 s (g) were obtained at 1000× magnification, and the sample at 600 s (h) was also obtained at 5000× magnification.

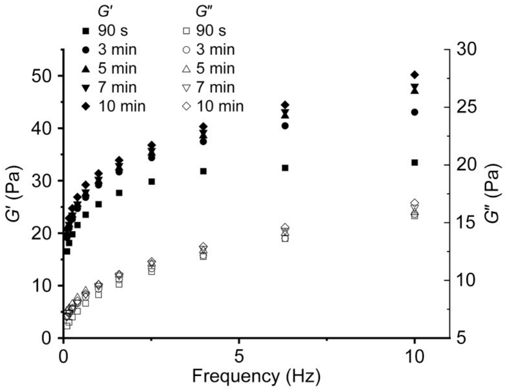

Fig. 4 Frequency sweep of ginger-juice milk curd at different clotting times. Solid symbols represent storage moduli (G') and hollow symbols represent loss moduli (G'').

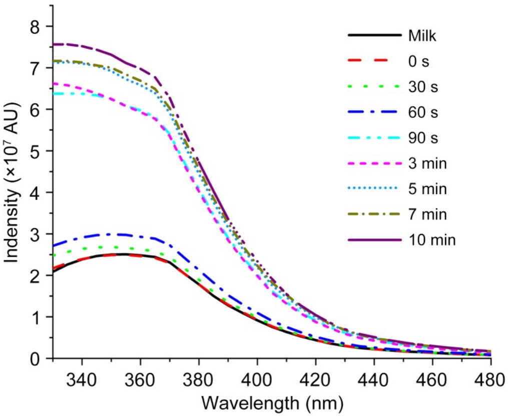

Fig. 5 Intrinsic fluorescence spectra of ginger-juice milk curd at different clotting times.

3.4 Analysis of fluorescence spectral characteristics

Intrinsic fluorescence was used to analyze the structural changes of casein during coagulation (Fig. 5). When casein undergoes hydrolysis by ginger protease, chromophores tryptophan, tyrosine, and phenylalanine are exposed to the solvent, leading to an increase of fluorescence intensity. The fluorescence spectra of milk and ginger curd at 0 s were similar, indicating that the curd components in ginger juice had not significantly changed at this time. The fluorescence intensity was in a low range before 60 s and reached a high level between 90 s and 10 min, resulting in a significant increase of intensity from 60 to 90 s. Therefore, we predicted that curd was initially formed at 90 s with significant changes of the tertiary structure of protein.

3.5 Analysis of flavor characteristics

3.5.1 Taste analysis

Results from the taste analysis of milk, ginger juice, and ginger-juice milk curd are shown in Fig. 6. The three samples did not have sourness, but the sweetness and bitterness were strong. Their aftertaste-bitterness and aftertaste-astringency were similar and at a low level. The taste of ginger-juice milk curd was similar to that of milk. The astringency and saltiness of ginger-juice milk curd were lower than those of ginger juice, which suggested that milk can neutralize and adjust the unwelcome taste in ginger juice, thereby making the product more popular with consumers.

Principal component analysis (PCA) was further carried out to extract the chemical sensors (Fig. 7). The contributions of the first and second principal components were 77.8% and 20.1% respectively, with a total of 97.9%, indicating that most of the information of samples was included and could be used for analysis. The ginger juice content was strongly correlated with saltiness, astringency, and aftertaste-astringency. Ginger juice was located at different positions from the other two samples with a large degree of dispersion. However, ginger-juice milk curd and milk were relatively close, indicating a high similarity in their taste, which is consistent with the results of the radar chart (Fig. 6).

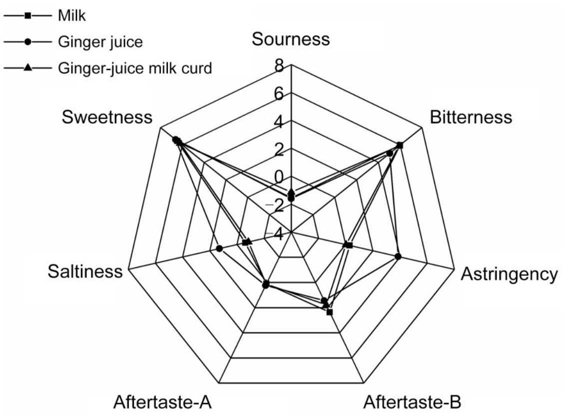

Fig. 6 Electronic tongue analysis of milk, ginger juice, and ginger-juice milk curd.

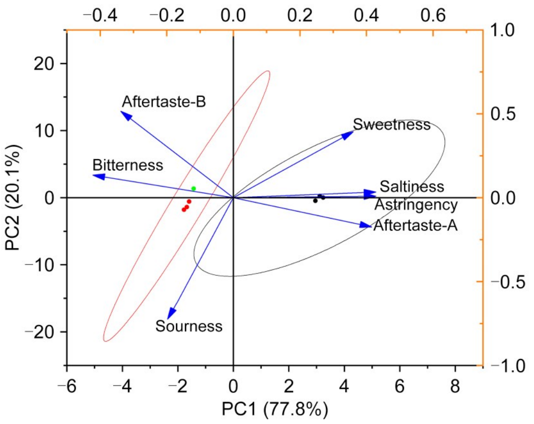

Fig. 7 Principal component analysis (PCA) of electronic tongue data. Each sample was tested in triplicate. The black dots represent ginger juice, red dots represent ginger-juice milk curd, and green dots represent milk.

3.5.2 Analysis of volatile compounds

Volatile compounds of ginger juice, milk, and ginger-juice milk curd with coagulation for 10 min were determined by gas chromatography-mass spectrometry (GC-MS) and the total ion flow diagrams are shown in Fig. S2. The volatile compounds in ginger juice were more abundant than those in milk and ginger-juice milk curd. The maximum peak was around 25 min for ginger-juice milk curd. The main volatile flavor compounds in ginger juice, milk, and ginger-juice milk curd are shown in Table 1. Sesquiterpenic hydrocarbons, including α-zingiberene, α-curcumene, β-terpinene, and camphene, were the main volatile flavor compounds in ginger juice. The main volatile components in milk were ketones, alcohols, and acids, including 2-heptanone, 2-nonanone, 2-methyl-2-nonanone, eucalyptol, and octanoic acid. The volatile flavor compounds in ginger-juice milk curd came mostly from ginger juice (α-zingiberene, α-curcumene, citral, and camphene) and milk (2-methyl-2-nonanone and octanoic acid). However, the volatile compounds in ginger-juice milk curd were not a simple combination of those in ginger juice and milk. In the process of coagulation, some volatile compounds disappeared and some new compounds were produced. For example, eucalyptol, which has antibacterial and anti-inflammatory functions, was detected in ginger juice and milk, but not in ginger-juice milk curd. β-Sesquiphellandrene, β-bisabolene, β-myrcene, β-linalool, citronellol, γ-elemene, caryophyllene, and octanoic acid were newly generated, serving as unique flavor compounds in ginger-juice milk curd. α-Zingiberene, found in both ginger juice and ginger-juice milk curd, is the main component of the essential oil of *Guarea kunthiana* A. Juss, and has antimicrobial properties, especially against Gram-positive and Gram-negative bacteria (Pandini et al., 2018).

Table 1 Relative contents of volatile flavor compounds found in ginger juice, milk, and ginger-juice milk curd with coagulation for 10 min

Compound	Relative content (%)		
	Ginger juice	Milk	Ginger juice milk curd
Citral	14.163±0.702	0.831±0.039	11.704±0.503
α-Zingiberene	9.708±0.498		22.706±1.102
α-Curcumene	7.684±0.403		4.486±0.197
β-Sesquiphellandrene			9.104±0.403
β-Terpinene	7.334±0.368		
Camphene	7.213±0.401		2.288±0.101
2-Heptanone		5.724±0.303	
2-Nonanone		4.118±0.203	
α-Pinene	4.067±0.196		
D-Limonene	2.642±0.103		0.304±0.010
Methylheptenon			2.305±0.103
β-Phellandrene	2.304±0.102		
β-Bisabolene			2.151±0.104
α-Phellandrene	2.003±0.105		0.868±0.038
Thujopsene	2.004±0.102		
β-Myrcene			1.914±0.092
Borneol	1.470±0.072		1.311±0.072
Octanoic acid		1.243±0.061	0.094±0.004
2-Methyl-2-nonanone		1.172±0.051	0.082±0.011
Copaene	1.093±0.051		
Eucalyptol	0.734±0.042	3.581±0.178	
β-Linalool			0.703±0.032
α-Cubebene	0.384±0.023		1.277±0.058
Citronellol			0.210±0.011
γ-Elemene			0.181±0.012
Caryophyllene			0.162±0.013
4-Carene	0.162±0.011		
3-Carene	0.143±0.012		
Decanoic acid			0.132±0.012
cis-α-Bisabolene			0.131±0.010
Ylangene	0.091±0.004		

3.5.3 Dynamic changes of volatile compounds in ginger-juice milk curd during coagulation

To further analyze the dynamic changes of volatile flavor compounds during coagulation, the curd coagulated from 0 to 10 min was monitored (Table 2). Approximately 70 volatile components, including ketones, alcohols, esters, olefins, and aldehydes, were detected apart from a few siloxanes produced by the extraction fiber. Alkenes had the highest content in the volatile components of ginger-juice milk curd, including mainly α-zingiberene, α-curcumene, β-sesquiphellandrene, and β-bisabolene. The content of these substances decreased with the increase of coagulation time, and remained unchanged after 90 s. Perhaps this was because the unique volatile substances in ginger juice were diluted when the milk and ginger juice were mixed, and their content stabilized until the curd was formed after 90 s. 2-Nonanone and octanoic acid are unique volatile flavor compounds in milk, and their contents remain almost unchanged during coagulation, indicating that ginger juice has a significant impact on the flavor of ginger-juice milk curd.

As one of the volatile components of ginger juice, citral has a pleasant lemon flavor. Its content did not obviously change within 90 s but increased by more

Table 2 Relative contents of volatile flavor compounds found in ginger-juice milk curd with different clotting times of 0, 30, 60, 90, and 600 s

Compound	Relative content (%)				
	0 s	30 s	60 s	90 s	600 s
α-Zingiberene	27.376±1.301	26.982±1.304	25.256±1.196	22.661±1.103	22.712±1.102
α-Curcumene	18.412±0.901	6.092±0.304	5.421±0.203	4.677±0.202	4.489±0.202
β-Sesquiphellandrene	12.864±0.601	12.256±0.598	10.721±0.504	9.012±0.401	9.104±0.402
β-Bisabolene	12.398±0.602	5.381±0.302	3.441±0.203	2.649±0.098	2.148±0.102
Citral	6.968±0.296	7.381±0.304	5.388±0.201	6.477±0.303	11.704±0.505
Copaene	1.501±0.070	1.442±0.073	1.448±0.082	1.222±0.061	
Camphene	0.964±0.052	0.910±0.041	1.411±0.074	2.323±0.105	2.292±0.104
β-Phellandrene	0.958±0.042	2.524±0.102	4.176±0.202	6.071±0.303	
Epizonarene	0.787±0.028				
β-Myrcene	0.612±0.030	0.741±0.042	1.343±0.064	1.722±0.085	1.913±0.094
α-Pinene	0.461±0.024	0.378±0.016	0.650±0.031	1.011±0.052	
Borneol	0.422±0.023	0.564±0.030	0.951±0.052	1.316±0.061	1.307±0.068
α-Phellandrene	0.370±0.018	0.402±0.021	0.687±0.030	0.981±0.052	0.867±0.038
Methylheptenon	0.359±0.020	0.718±0.043	1.456±0.074	1.340±0.062	2.302±0.104
Seychellene	0.262±0.014				
Pulegone	0.238±0.010	0.410±0.023			
D-Limonene	0.212±0.018	0.221±0.012	0.210±0.023	0.591±0.030	0.301±0.012
β-Linalool	0.182±0.012	0.274±0.014	0.440±0.021	0.593±0.032	0.702±0.034
α-Cadinol	0.151±0.013	0.063±0.003	0.071±0.003		
Ylangene	0.129±0.012	0.127±0.007	0.110±0.005		
Citronellol	0.102±0.013	0.132±0.006	3.482±0.201	4.679±0.201	0.212±0.012
α-Cubebene	0.081±0.004	0.057±0.003	0.064±0.003	0.038±0.002	1.282±0.063
D-Citronellal	0.074±0.003	0.078±0.004	0.278±0.010	0.382±0.020	0.172±0.009
4-Carvomenthenol	0.062±0.003	0.091±0.004	0.140±0.007	0.191±0.012	0.214±0.009
τ-Cadinol	0.052±0.003				
Octanoic acid	0.044±0.002	0.047±0.003	0.072±0.003	0.056±0.002	0.087±0.004
Bornyl acetate	0.041±0.002	0.052±0.003	0.058±0.003	0.141±0.007	0.080±0.004
2-Undecanone	0.042±0.002	0.052±0.002	0.060±0.003	0.081±0.004	0.081±0.004
2-Nonanone	0.172±0.006	0.146±0.008	0.162±0.007	0.181±0.009	0.164±0.007
Decanoic acid				0.136±0.007	0.131±0.007
γ-Elemene				0.177±0.009	0.178±0.009
Caryophyllene				0.162±0.007	0.162±0.007
cis-α-Bisabolene				0.150±0.007	0.127±0.006

than 60% after 10 min. The contents of β-myrcene, camphene, and α-cubebene also increased. Jayaprakasha et al. (2012) demonstrated that the active principles of kumquat volatile oil had significant radical scavenging activity and great potential for the prevention of cancer. D-Limonene and β-myrcene, major compounds in kumquat volatile oil, were also found in ginger-juice milk curd. In addition, D-limonene can be used as an antioxidant in food (Jimenez et al., 2012). Terpenes such as β-myrcene, which was one of the unique flavor compounds of ginger-juice milk curd, have strong antioxidant properties (Pernice et al., 2009).

In addition to the volatile compounds of ginger juice and milk, ginger-juice milk curd has its own unique volatile compounds, such as decanoic acid, γ-elemene, and caryophyllene. Decanoic acid is a fatty acid that has a particularly unpleasant smell. It was produced when the curd was basically formed. If it can be eliminated or decreased, the quality of ginger-juice milk curd will improve. γ-Elemene contributed a pungent fragrance and is an effective anti-cancer ingredient. Caryophyllene has anti-inflammatory (Basha and Sankaranarayanan, 2016) and antifungal properties. As a result, ginger-juice milk curd not only offers unique flavor characteristics but also potential health benefits, making it a valuable addition to the diet.

4 Conclusions

This study explored the changes in physiochemical, structural, and flavor characteristics during the coagulation of ginger-juice milk curd. Ginger protease, which is the main coagulation factor in ginger juice, showed relatively high activity before 60 s. It can hydrolyze κ-casein which is in the outer shell of casein micelles. Then, α_s- and β-casein, which are inside the micelles, were released and further hydrolyzed into small molecules, disrupting the stability of micelles. Free Ca^{2+} combined with casein to form a 3D network structure. There was a significant increase of intensity from 60 to 90 s, indicating that curd was initially formed at 90 s with significant changes of tertiary protein structure. As the clotting time went by, the content of Ca^{2+} in whey, the content of soluble protein, and the activity of ginger protease gradually decreased, while the hardness, adhesiveness, and WHC increased along with a more compact gel structure. The taste of the ginger milk curd was similar to that of milk, and could neutralize the astringency and saltiness of ginger juice. The main volatile flavor compounds of ginger-juice milk curd were α-zingiberene, α-curcumene, β-sesquiphellandrene, and β-bisabolene, whose contents decreased at the beginning but remained unchanged after 90 s. Some new flavor compounds, such as γ-elemene and caryophyllene, were produced after coagulation. In summary, the physiochemical, structural, and flavor characteristics underwent significant changes during coagulation. Further analyses of the composition and content of various caseins and observations of 3D structure by confocal laser scanning microscopy are being carried out in our laboratory.

Data availability statement

The dataset used or analyzed during the current study is available from the corresponding author on reasonable request.

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Author contributions

Haifeng PAN performed the experimental research and data analysis, and wrote and edited the manuscript. Wenna BAO contributed to the study design, data analysis, and reviewing and editing of the manuscript. Yi CHEN and Hongxiu LIAO performed data analysis and figure organization. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Haifeng PAN, Wenna BAO, Yi CHEN, and Hongxiu LIAO declare that they have no conflicts of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Supplementary information

Materials and methods; Figs. S1 and S2