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Research Article

Effect of Carrot Juice on the Texture Properties, Rheology, and Microstructure of Yoghurt

Li-Ying Bo, Zhi-Qin Pan, Tian-Ying Sun, Guo-Jun Du, Duo Zhang, Zhi-Qiang Song, Chun-Li Song, and Ren Jian

Faculty of Food Quality and Safety, Qiqihar University, Qiqihar 161006, China

Correspondence should be addressed to Chun-Li Song; songchunlilily@sina.com

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In the present study, carrot juice prepared was added into skimmed milk before fermentation to produce 10% and 20% (w/w) carrot-fortified skim milk. The influence of carrot juice on the lactic acid content, chemical composition, syneresis, texture, rheology property, and microstructure was evaluated along with a potential of carrot juice as an ingredient with rich dietary fiber and a firm structure with interaction of milk protein and a complex matrix under acidification. The results showed that the pH of yoghurt significantly ($P < 0.01$) reduced with carrot juice addition, minimizing fermentation time by nearly 1 h and brought about an enhanced postacidification during the storage period while titratable acidity increased. Moreover, carrot juice addition had no significant effect on ferric reducing power and phenolic content ($P > 0.05$). With 10% and 20% level carrot juice added, the total phenolic content (mg GAEkg^{-1}) increased (37.86 and 38.92, respectively) compared with control (36.91) ($P < 0.05$). Besides, the addition of carrot juice also showed reduced syneresis, increased apparent viscosity and viscoelastic modulus, stable texture, and a refined microstructure. Overall, this study confirmed that carrot juice has a potential as natural and nutrient composition to obtain firm and compact texture and enhance the beneficial quality of yoghurt.

1. Introduction

Currently, yoghurt is viewed as one of the most popular fermented dairy products and it has obtained widespread well-being with benefits of energy and nutrition value [1]. The yoghurt product by microbial fermentation of bovine milk is produced by using probiotic bacteria consisting of a combination of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. During fermentation, these probiotics are responsible for conversion of lactose into lactic that minimizes the pH value. When the pH of fermentable milk attains the isoelectric point of casein in the milk, casein starts to interact with each other, resulting in some poor-quality attributes, such as loss of solubility and the formation of very specific three-dimensional structure [2, 3]. So, improving quality attributes of the yoghurt product is the concern of human research. However, adding food additives including pectin, xanthan gum, starches, and gelatin, among others into yoghurt products is unacceptable for consumers in some countries because humans prefer to natural food products without hydrocolloid stabilizers [4–6]. In addition, yoghurt production without additives is also in line with the increasing demand for "green label" products [7]. Thus, it is an inevitable trend to use natural ingredient instead of food additives aiming to improve yoghurt quality and enhance the effectiveness of nutritional values. Apple pomace addition with formulation compensation in yoghurt processing had resulted in an acceptable product and enhanced the extent of yoghurt firmness [2].

Generally, edible carrot portion contains less water, protein, total carbohydrates, and lipids. Moreover, carrot is also classified as a vitaminized food in that carrot also includes other primary nutritional components; it is rich in dietary fibers (approximately 32 g), vitamins (β-carotene), ascorbic acid, and minerals (e. g. iron, copper, sulfur, magnesium, manganese, and potassium) [8] but lacks in high-quality protein and fat. While yoghurt is rich in protein, lipid, and calcium, it is deficient in vitamin, mineral, and dietary fibers [9]. Therefore, a combination of yoghurt and carrot juice may enhance nutrition [10] and have a firming texture of set-type yoghurt. Yoghurt has been viewed as a carrier of dietary fiber and other nutritional components in a variety of studies. From the aspect of nutritional value, dietary fiber-rich products such as carrot juice and apple pomace may possess a host of functional characteristics, such as fat adsorption, water hydration, and viscosifying and texturizing properties [11]. Tanje et al. found that dietary fiber added into yoghurt by gelling-texturizing and water holding capacity can provide positive contribution to the firm texture and shelf life of yoghurt [12]. The results from a previous study also proved that the addition of the carrot juice and stabilizer into yoghurt resulted in improved nutritional component and suppressed syneresis but did not specify which ingredient played a leading effect [13].

The prime purpose of this study was to characterize the functionality of carrot juice as a natural ingredient for dairy products and evaluate the influence of carrot juice on physicochemical, texture and rheological properties, and functional (total phenolic and ferric reducing power) properties of fermented milk products during cold storage (21 days).

2. Materials and Methods

2.1. Materials and Reagents. In this study, the skimmed bovine milk powder was bought from Fronterra Trading (Shanghai) Co. Ltd. Carrot was purchased from a local supermarket (Qiqihar, Heilongjiang, China). 4-hydroxybiphenyl and anhydrous lithium lactate were obtained from Shanghai Aladdin Co. Ltd. and Sigma-Aldrich Co. (St. Louis, MO, USA), respectively. Dvs starter (YO-MIX 499) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckiisubsp. bulgaricus* was obtained from Danisco GmbH. (Beijing, China). The rest of the chemical reagents used were of analytical grade. Deionized water was used in the preparation of carrot juice and all solution.

2.2. Preparation of Carrot Juice and Yoghurt Samples. Fresh carrots were cleaned thoroughly, peeled and mixed with deionized water in a ratio of 1 : 1, grounded by a high-speed homogenizer (Hangzhou Joyoung Co Ltd., Hangzhou, China) into juice for 1 min at 20°C, and then centrifuged to collect carrot juice. The skimmed milk powder and sucrose were dissolved in pure water at contents of 100 g/kg and 60 g/kg, respectively. Afterwards, the carrot juice was added to the skimmed milk at two set concentrations of 1 : 9 and 2 : 8 (w/w) and fully mixed for 10 min with a magnetic stirrer before heating. The skimmed milk without carrot juice was named as control. Set-type yoghurt was prepared according to the method described by Lucey and Lee with slight modifications [14]. Carrot-milk mixture was heated at 90°C for 10 min in a water bath and then cooled to approximate 42°C. They were inoculated with the DVS starter at a level of 0.06 g/kg milk according to proposal of the supplier, poured into a 50 mL beaker under aseptic conditions, and then fermented at 42°C for about 5 h. Yoghurt samples were stored in a refrigerator at 4°C for 24 h to analyze their chemical composition, microstructure, and textural and rheological properties at day 1 (overnight), 14, and 21.

2.3. Chemical Composition of Yoghurt and Carrot Juice. The fat, protein, and ash contents of yoghurt were detected according to AOAC methods No. 905.02, No. 991.20, and No. 945.46, respectively [15]. Protein contents of yoghurt were estimated by a conversion factor (6.38) of nitrogen to protein.

The total solids levels of yoghurt and carrot juice were detected as described in AOAC methods No. 990.20 and 920.151 [15].

2.4. Chemical Analysis of Yoghurt and Carrot Juice

2.4.1. Total Phenolic Content Measurement. The total phenolic content of carrot juice was detected according to the Folin–Ciocalteu method after extraction with 30 mL aqueous ethanol (80%, containing 1% HCl), agitating for 2 h at 50°C and filtering according to the described method [16]. Calibration curves were prepared according to the following concentration contents of gallic acid: 25, 50, 75, 100, 125, and 150 μg/mL. The 100 μL extract and 750 μL of Folin–Ciocalteu solution were mixed, allowed to stand for 5 min, and then gently mixed using 750 μL 6% sodium carbonate (w/v). After standing for 40 min, absorbance was determined at 725 nm with a UV/Visible spectrophotometer (Shimadzu Co. Ltd., Kyoto, Japan) and assayed results were expressed as mg of gallic acid equivalent per 100 g of carrot juice.

The total phenolic was extracted by the provided method in Wallace and Giusti's literature [17]. Yoghurt samples were blended with 30 mL acidified methanol (containing 0.1% HCl) for 2 min and then centrifugated for 15 min at 3500 rpm. The supernatant was mixed with 50 mL of acidified methanol, and total phenolic was measured as described above.

2.4.2. pH Measurement. The pH was determined with a digital pH meter (Mettler, Toledo, FE28 pH, Shanghai, China) at room temperature after a rectification using the standard buffer solvents with pH values of 4.01, 6.86, and 9.18, respectively. Total protein content, titratable acidity, and total solids of the yoghurt samples were detected according to the AOAC [18] method using an automatic titration apparatus.

2.4.3. Ferric Reducing Power Measurement. The ferric reducing power of carrot juice was detected using the previous method [19]. The 100 μL carrot juice or yoghurt extracts were blended with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL 1% potassium ferricyanide and incubated at 50°C for 20 min in a water bath. 2.5 mL 10% trichloroacetic acid was added to the above mixture and centrifuged at 3500 rpm for 10 min. The upper layer (2.5 mL) and distilled water

(2.5 mL) were mixed with freshly prepared ferric chloride reagent (0.1%, 2.5 mL). Absorbance at 700 nm was determined.

2.5. Rheological Measurement of Yoghurt. Viscoelastic and viscosity behaviours of the yoghurt samples were measured by a Bohlin Gemini II Rheometer (Malvern Instruments Limited, Worcestershire, UK) with a cone-plate geometry (cone angle 4°, gap 0.15 mm and diameter 40 mm) at room temperature, according to previous reference methods [20, 21]. The yoghurt samples were allowed to return to room temperature and gently stirred 10 times at 2-3 s per rotation with a tablespoon aiming to achieve a homogeneous state before measurement. Elastic and viscous modulus (G' and G'') were detected using the cone-plate geometry, frequency sweeps in 0.5% strain and 0.1–10 Hz, and shear rates of 0.1–10/s (within the linear viscoelastic region).

2.6. Texture Analysis of Yoghurt. The yoghurt texture was determined using a stable Micro Systems Texturometer (Texture Technologies Corp, New York, USA) equipped with a 5 kg load cell as the previous research method [22]. The samples (50 mm diameter 50 mm height) held in the glass containers were required to equilibrate to room temperature before putting the containers on the console. The parameters were modified: head velocity speed of 1.0 mm/s, cylinder probe of 35 mm diameter, surface trigger force of 10 g, and penetration distance of 30 mm. The samples were replaced in 125 mL containers with 70 mm height and 64 mm diameter. Hardness, cohesiveness, adhesiveness, and springiness were calculated by the XT.RA Dimension ver. 3.7 program.

2.7. Microstructure Observation and Syneresis Analysis of Yoghurt. The microstructure of the samples was evaluated using the S-3400N Scanning Electron Microscope (Hitachi High-Technologies Co., Tokyo, Japan) following a previous method performed with some changes [22]. A cube (4 mm × 4 mm × 3 mm) was segregated from about 1 cm under the surface and fixed using 0.1 mol/L phosphate buffer (pH 6.8) including 25 g/kg glutaraldehyde for 24 h. The specimens were washed with 0.1 mol/L phosphate buffer (pH 6.8) three times for 10 min each time and dehydrated with different concentrations of ethanol (50–90%, 15 min each time). Afterwards, the specimens were dipped in tert-butyl alcohol, absolute ethanol solutions (1 : 1, v/v), and tert-butyl alcohol (15 min) and then lyophilized in liquid nitrogen (48 h). The treated specimens were placed on the holders in a Hummer VI sputtering system (Matsushita Electric Industrial Co., Osaka, Japan) with an adhesive carbon film bonding and with gold spraying and then observed and photographed with a magnification of ×2000 under the scanning electron microscope.

Syneresis of the samples was detected according to the method described by Keogh and O'Kennedy [23]. Syneresis was counted as the supernatant (whey) produced after lower speed centrifugation (at 222 ×g for 10 min) of 20 g yoghurt samples. The supernatant was dumped and weighed right away. The syneresis parameter of these samples was expressed as weight %.

2.8. Statistical Analysis. All data derived from analyses were obtained from at least three independent experiments and reported as means ± standard deviations. Differences between the multiple groups were carried out using one-way analysis of variance (ANOVA) and two-way analysis of Spearman. All analyses and the reported data were carried out with Duncan's analysis and Spearman's analysis SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) and MS Excel 2003 (Microsoft Corporation, Redmond, WA, USA).

3. Results and Discussion

3.1. Chemical Composition of Carrot Juice and Milk Used. The freshly prepared carrot juice's total carbohydrates, moisture, total solids, and total phenolic contents assayed on weight basis were 6.0, 91.7, 7.6%, and 46.3%, respectively, and similar to previously reported values on carrot juice by Negri Rodríguez Livia et al. [8]. The milk water, total solids, fat, protein, and ash contents were 86.8, 13.2, 3.2, 3.0, and 0.8, respectively, and consistent with those reported for cow milk [24].

3.2. Chemical Composition of Yoghurt. In this study, crude protein, total solids, fat, and ash contents (%, w/w) of these samples were in the range of 2.7–3.1%, 15.4–16.6%, 2.8–3.3%, and 0.70–0.73%, respectively (Table 1). This meant that chemical composition of these yoghurt samples was significantly ($P < 0.01$) affected by carrot juice addition. Being accompanied by carrot juice increase, fat content decreased while the moisture level of yoghurt increased. With carrot juice doses increased, crude protein content significantly lowered, but the difference was insignificant ($P > 0.05$) between yoghurt samples containing 15% and 20% carrot juice. The ash level of yoghurt samples also reduced as carrot juice addition levels increased even though ash values of yoghurt samples including 10% and 20% carrot juice were similar.

Changes of proximate composition in yoghurt samples were responsible for composition difference between used carrot juice and milk. Carrot juice addition had a certain dilution influence on composition of yoghurt, and this was dependent on high moisture levels of carrot juice. Information on yoghurt composition is mandatory from quality inspection department. According to the description in Codex for yoghurt standard, in the present study, yoghurt samples are satisfied with these requirements [25].

3.3. Total Phenolic and Ferric Reducing Power. Some factors e.g., sanitizing reagent contamination, protein metabolized by bacteria, specific fodder fed by cattle, and deliberate addition as particular functional constituents result in phenolic compound occurrence in dairy products [26]. Total phenolic level increase and correspondingly ferric reducing power increase could be expected to be present in yoghurt

Table 1: Chemical composition of yoghurt with different carrot juice addition doses.

Composition	Control	10% CJ	20% CJ
Moisture content (%)	81.56 ± 0.04^{c}	82.28 ± 0.06^{b}	83.73 ± 0.05^{a}
Fat (%)	3.31 ± 0.03^{a}	3.07 ± 0.02^{b}	2.79 ± 0.04^{c}
Total solids (%)	16.58 ± 0.05^{a}	15.89 ± 0.06^{b}	15.36 ± 0.04^{c}
Crude protein (%)	3.11 ± 0.03^{a}	2.89 ± 0.05^{b}	2.73 ± 0.02^{c}
Ash (%)	0.73 ± 0.02^{a}	0.71 ± 0.01^{a}	0.70 ± 0.01^{a}
Ferric reducing power (mg GAE kg^{-1})^{NS}	112.41 ± 2.87^{a}	110.74 ± 2.54^{a}	108.23 ± 3.52^{a}
Total phenolic content (mg GAE kg^{-1})^{NS}	36.91 ± 1.06^{a}	37.86 ± 1.13^{a}	38.92 ± 1.08^{a}

The data are means ± SD (standard deviations). CJ: carrot juice; GAE: gallic acid equivalent; NS: nonsignificant. Different lowercase letters a-c show significance by the one-way ANOVA test ($P < 0.05$).

samples with higher contents of carrot juice. Because of the phenolic level in carrot juice used being relatively low, total phenolic content and ferric reducing power are enhanced though carrot juice fortification was slight. This is partly due to the binding of phenolic compound with milk casein before gel formation during fermentation. The present phenomenon is consistent with the previous study. When adding the grape seed extract into milk before fermentation, total phenolic content and ferric reducing power had no significant change [26, 27]. The total phenolic content in carrot should be related to cultivar, postharvest handling, and processing methods [8]. Hence, there is possible potential to improve yoghurt quality with antioxidant, dietary fibers, and provitamin A supplementation for other possible phytonutrients (e.g., ascorbic acid, tocopherols, phenolic, and fibers) derived from carrot juice.

3.4. pH and Titratable Acidity of Yoghurt. Whether the added carrot juice had a certain effect on the yoghurt product was important to dairy production, taking into account that the fermentation time is prime to actual production for dairy enterprises. Therefore, the pH and titratable acidity were detected in yoghurt fermentation processing. The results exhibited that the pH value and titratable acidity showed corresponding decreasing and increasing trend during fermentation processing, respectively, being accompanied by the extended fermentation time (Table 2). At 4-h point of fermentation, distinct pH drop and titratable acidity rise were observed. At the end of fermentation processing, pH was close to 4.6. And what is more, the additional carrot juice had a significant influence on dairy products because it leaded to an obvious pH drop or titratable acidity rise for yoghurt samples with carrot juice. Therefore, it is supposed that the additional carrot juice had promoted effect on LAB and resultantly led to a higher increase in acidity for yoghurt samples with carrot juice. After the added carrot juice, at 4-h point of fermentation, the pH and titratable acidity of carrot-milk mixtures reached to the value of yoghurt without carrot juice at the 5-h (end-point) of fermentation. More specifically, the additional carrot juice resulted in reduced fermentation time of about 1 h. Moreover, compared to yoghurt containing 10% carrot juice, the addition of higher level of carrot juice resulted in much acid substance. In general, these phenomena clearly showed that the addition of carrot juice did not prevent yoghurt fermentation processing but promoted it, indicating potential application of carrot juice for yoghurt fermentation processing. The two previous examples of research had been demonstrated to shorten the fermentation time of yoghurt by adding pineapple waste powder and the *Siraitia grosvenorii* fruit extract [27, 28]. Carrot juice included soluble dietary fibers such as viscous polysaccharides, which might be used as facilitating factors to promote LAB proliferation during fermentation processing and human digestive tract. Another study had also found that dietary fibers could increase probiotic growth by adding dietary fiber in vegetables in yoghurt products [29]. These mentioned results provided strong scientific support for our present study.

Moreover, the pH value of all samples minimized from 4.4 to 4.2 during the 21-day storage period ($P < 0.01$) (Table 3), but the largest *pH drop occurred in the first* 7-day cold storage stage. The samples fortified with carrot juice exhibited lower pH compared to the control samples during most of the cold storage period. At the same time, the titratable acidity of the yoghurt samples increased in similar tendency with the decrease in the pH value. The pH and the titratable acidity had slight changes for all samples at the end of the 21-day cold storage period ($P > 0.05$). The obvious drop of pH in the first 7-day cold storage stage has similar results with the previous study of adding vegetables and fruit byproducts into yoghurts [30]. Consequent rise of lactic acid introduced by highly active starters led to this decrease at the initial stage of cold storage. Ingredient acids found in carrot juice can cause a positive effect on the pH and acidity of yoghurt.

3.5. Postacidification of Yoghurt with Carrot Juice. During yoghurt storage, pH reduction or titratable acidity enhancement adequately took on the postacidification phenomenon. The results displayed in Table 3 showed that all detected samples had postacidification phenomenon because of observing pH reduction and titratable acidity increase at different times of yoghurt storage. According to the analysis method described in two previous literatures [31, 32], a 2-way ANOVA was carried out. Based on the results of the study listed in Table 3, the correlation of storage time and the amount of carrot juice significantly ($P < 0.05$) affected variation of pH and titratable acidity value. In the situation of the same storage time, carrot juice addition obviously brought about the changes of the pH value and the titratable acidity value. The results showed that yoghurt samples with 10% and 20% carrot juice supplemented always manifested a lower pH level and a higher titratable acidity

Table 2: Changes in the pH values and titratable acidity of these samples during fermentation processing.

Parameters	Fermentation time (h)	Control	10% CJ	20% CJ
pH	0	6.56 ± 0.03^a	6.55 ± 0.01^a	6.45 ± 0.01^a
	1	6.52 ± 0.02^b	6.50 ± 0.01^a	6.44 ± 0.03^a
	2	5.81 ± 0.01^c	5.54 ± 0.04^b	5.70 ± 0.01^b
	3	5.14 ± 0.05^d	5.03 ± 0.09^c	5.03 ± 0.06^c
	4	4.88 ± 0.01^e	4.69 ± 0.04^d	4.72 ± 0.05^d
	5	4.68 ± 0.03^f	4.52 ± 0.02^e	4.43 ± 0.01^e
Titratable acidity (lactic acid%)	0	0.17 ± 0.02^F	0.19 ± 0.03^F	0.20 ± 0.02^F
	1	0.20 ± 0.01^E	0.24 ± 0.04^E	0.27 ± 0.01^E
	2	0.29 ± 0.02^D	0.34 ± 0.01^D	0.35 ± 0.01^D
	3	0.37 ± 0.01^C	0.51 ± 0.04^C	0.54 ± 0.04^C
	4	0.45 ± 0.03^B	0.72 ± 0.03^B	0.73 ± 0.01^B
	5	0.71 ± 0.02^A	0.82 ± 0.02^A	0.87 ± 0.03^A

The data are expressed as means ± SD (standard deviations). The analyses were performed three times. CJ: carrot juice. Different lowercase letters a-f and capital letters A-F show significance by the two-way Pearson test ($P < 0.01$).

Table 3: Changes in the pH and titratable acidity of these yoghurt samples during different cold storage periods.

Parameters	Storage time (days)	Control	10% CJ	20% CJ
pH	1	4.37 ± 0.01^a	4.35 ± 0.02^a	4.32 ± 0.02^a
	14	4.23 ± 0.03^b	4.20 ± 0.01^b	4.21 ± 0.01^b
	21	4.17 ± 0.01^c	4.16 ± 0.02^c	4.19 ± 0.02^c
Titratable acidity (lactic acid%)	1	0.77 ± 0.02^B	0.88 ± 0.01^B	0.91 ± 0.01^B
	14	0.85 ± 0.01^B	0.92 ± 0.02^A	0.95 ± 0.01^A
	21	0.84 ± 0.01^A	0.93 ± 0.01^A	0.96 ± 0.01^A

The data are reported as means ± SD. The experiments were carried out three times. Different lowercase letters a-c and different capital letters A-B show significance by the one-way ANOVA test ($P < 0.05$).

level, as compared to control samples without carrot juice. In general, after the 21-day storage period, yoghurt samples with carrot juice had a proximate titratable acidity value, which increased more obviously than the control sample. The carrot juice level was a significant influencing factor for titratable acidity and postacidification of yoghurt samples. It is well known that lactic acid is prime organic acid produced in yoghurt products. The obtained results also verified that during storage both carrot juice concentration and storage time had an effect on the amount of lactic acid of these samples. When prolonging storage time, lactic acid contents of these yoghurt samples also enhanced significantly. Similarly, addition of carrot juice was responsible for an increased lactic acid level in yoghurt samples with carrot juice. What is more special is that titratable acidity ranged from 0.70 for the control sample to 0.96 titratable acidity for samples with 20% carrot juice. These data confirmed that carrot juice addition resulted from lactic acid production during the 21-day storage period. The lactic acid values observed in our study were lower than previously reported [33]. From flavor development perspective, titratable acidity should be controlled within certain limits because of consumer preferences. Titratable acidity of this study was above the minimum limit of 0.60 recommended by Codex [25].

During yoghurt storage, further proliferation of *S. thermophilus* and *L. bulgaricus* also influenced lactic acid formation due to lactose fermentation that is considered as the prime reason of yoghurt products postacidification [15]. Therefore, postacidification is an inevitable event in the storage period of yoghurt, including viable LAB. In the current study, these yoghurt samples showed a further increase in titratable acidity during storage. In a previous study of Patel and coauthors, high-amylose maize starch and inulin could enhance lactic acid production of a set-type yoghurt fermented only with one stain (*L. casei* or *L. acidophilus*) [34]. Another study found that starches and yam mucopolysaccharides were mixed to be able to promote the metabolism and proliferation of LAB, which resulted from the fermentation and hydrolysis of lactose [35]. Our study found that carrot juice endued with the stored yoghurt samples with carrot juice owning higher lactic acid levels (Table 4), indicating the phenomenon being consistent with these above studies. It was suggested that carrot juice could be used to enhance the postacidification of yoghurt samples.

3.6. Texture and Rheological Properties of Yoghurt Containing Carrot Juice. The textural characteristics of these samples are shown in Table 5. From a perspective of these obtained data, a combination of carrot juice and stored time mainly had a significant effect on hardness, cohesiveness, and adhesiveness values. But springiness values were not affected by carrot juice and stored time. In terms of detail, extended storage time was responsible for enhanced hardness, cohesiveness, and adhesiveness values, whereas carrot juice (especially for the yoghurt sample with 20% carrot juice) also brought about value increases for these indices. One of the most key parameters of yoghurt products is hardness. The hardness value of mixtures of yoghurt with 10% and 20% carrot juice all increased by 105 g, showing these samples

Table 4: Changes in lactic acid contents of yoghurt samples manufactured during cold storage.

Storage time (days)	Control	10% CJ	20% CJ
1	12.80 ± 0.21^{b}	13.51 ± 0.18^{b}	14.21 ± 0.20^{b}
14	14.61 ± 0.17^{a}	16.23 ± 0.22^{a}	16.68 ± 0.15^{a}
21	15.03 ± 0.14^{a}	16.54 ± 0.16^{a}	16.82 ± 0.13^{a}

Results shown as mean ± standard deviations of different addition amounts of carrot juice in yoghurt samples during cold storage. $^{a, b}$Means followed after the values in a row are significantly different ($P < 0.05$).

with the carrot juice indeed formed a firm texture. Moreover, the hardness of yoghurt samples with carrot juice was higher than that of the control sample with the same storage time. Therefore, it can be concluded that carrot juice played a positive effect on the hardness of milk mixtures. The two past studies also showed that some contribution of food stabilisers resulted in the increased hardness and adhesiveness of yoghurt [36, 37]. Normally, the hardness of yoghurt is related to the content of total solids [38]. However, yoghurt samples adding carrot juice only slightly increased total solids content (Table 3). Adding carrot juice caused an obvious effect on textural quality of mixtures of milk fortified with 10% and 20% carrot juice. This is an implication that the current strategy adding carrot juice into the yoghurt product (not for the purpose of enhancing total solid content) in this way can indeed make yoghurt capable of a stable and rigid texture.

Three rheological parameters such as elastic, viscous moduli, and apparent viscosity were determined and compared for all samples stored for 1, 14, and 21 days of cold storage, in order to analyze the relationship between the addition level of carrot juice and value changes of these indices. The present study only provided the data for all samples with cold storage of 14 days (Figure 1). The results demonstrated that yoghurt samples containing 20% carrot juice were capable of higher viscosity values than that of the control and 10% carrot juice samples. Simultaneously, the same phenomenon was observed in fortified yoghurt samples when G'/G'' values of these samples were compared. In general, the control and yoghurt with 20% carrot juice exhibited the lowest and highest parameter values, respectively, implying that the addition dose level of carrot juice resulted in enhanced rheological properties for these prepared yoghurt samples. In addition, G' values of yoghurt fortified with 10% and 20% carrot juice were much greater than G'' values at the evaluated frequencies, revealing that they behaved like solid properties [39]. Yoghurt with 10% carrot juice had the proximate viscous modulus but higher elastic modulus compared to control. Yoghurt samples fortified with 20% carrot juice displayed the highest elastic and viscous modulus. This took on the effect of the carrot juice on yoghurt quality. The TPA results exhibited that all yoghurt samples had different textural features. Mixtures of milk containing 10% carrot juice and the control had similar texture index values ($P > 0.05$). Yoghurt with 20% carrot juice showed lower cohesiveness, higher hardness, and adhesiveness ($P < 0.05$) but proximate springiness ($P > 0.05$) compared to control without carrot juice and yoghurt with 10% carrot juice.

Various types of food additives are found in yoghurt products in order to improve rheological properties of yoghurt. The two previous studies reported that casein in milk was cross-linked with transglutaminase to form protein polymers, resulting in enhanced rheological texture [40, 41]. When enzymatic cross-linking treated milk protein, increased G'/G'' values were found in yoghurt samples [42]. A past study also showed that adding gelatin into camel milk could increase G'' values of acidified camel milk [43]. At the same time, the other study demonstrated that the G' value was enhanced by mixing gelatin with sodium caseinate [44]. Additionally, some researchers found that the values of three evaluated indices (viscosity and G'/G'' moduli) increased when cross-linked gelation was introduced into set-type skimmed yoghurt [45]. In this study, adding carrot juice to yoghurt samples meant that soluble polysaccharides were present in yoghurt. It is reasonable that carrot juice-fortified yoghurt samples had the trend of increasing index values.

3.7. Microstructure and Syneresis of Yoghurt with Carrot Juice. Microstructure analysis can be used to better explain the results from textural and rheological analysis [46]. Microstructural features of these samples were also identified. The micrograph of a continuous flocculent protein structure was observed (Figure 2 1 d). The results indicated that when carrot juice was added to fermented milk, the particles from carrot juice mixed into the three-dimensional network structure; moreover, a network of thickening protein strands was founded in the fortified fermented milk samples. The yoghurt including 20% carrot juice exhibited a consistent network of protein resulting from the embedded carrot juice particles (Figure 2 14 d). As the levels of carrot juice increased to 20%, the network showed less compact. The micrographs of the yoghurt samples without carrot juice are consistent with the control in past studies [47, 48]. The yoghurt with carrot juice made about different structure from that of yoghurt containing inulin and pectin. The protein network of the yoghurt with carrot juice showed more pores compared with the network of fermented milk with exopolysaccharides produced lactic acid bacteria [49]. A relatively stable protein network with carrot juice particles trapped in the yoghurt sample with 10% carrot juice has not been found in other studies. Such kind of microstructure can correlate greatly with results of texture analysis, and the highest similarity that this sample showed is compared with the yoghurt samples containing other levels of carrot juice.

Syneresis is a main defect in fermented dairy products that may affect acceptability and the shelf life due to undesirable appearances. Carrot juice addition obviously

Table 5: Textural indices of carrot juice enriched yoghurt samples during cold storage.

Sample	Storage time (days)	Hardness	Adhesiveness	Springiness	Cohesiveness
Control	1	95.6 ± 5.7^{f}	792.3 ± 137.6^{d}	0.92 ± 0.03^{a}	0.32 ± 0.01^{e}
	14	126.3 ± 15.9^{e}	1087.4 ± 210.6^{c}	0.89 ± 0.01^{ab}	0.34 ± 0.01^{de}
	21	178.5 ± 8.2^{cd}	1300.7 ± 128.6^{c}	$0.85 \pm 0.02b^{cd}$	0.35 ± 0.02^{cd}
10% CJ	1	169.7 ± 10.6^{d}	1856.3 ± 123.5^{b}	0.83 ± 0.02^{d}	0.37 ± 0.01^{c}
	14	191.3 ± 5.9^{c}	1878.6 ± 153.6^{b}	0.83 ± 0.01^{d}	0.40 ± 0.03^{b}
	21	220.4 ± 6.3^{b}	2000.7 ± 124.5^{ab}	0.82 ± 0.02^{d}	0.41 ± 0.01^{b}
20% CJ	1	247.9 ± 4.1^{a}	2117.9 ± 67.3^{ab}	0.84 ± 0.02^{cd}	0.42 ± 0.02^{b}
	14	254.6 ± 8.3^{a}	2210.3 ± 100.4^{a}	0.88 ± 0.01^{abc}	0.45 ± 0.03^{a}
	21	260.1 ± 4.8^{a}	2241.5 ± 87.2^{a}	0.90 ± 0.03^{a}	0.47 ± 0.02^{a}

Data are reported as mean ± standard deviations of each supplement level of carrot juice (CJ) in yoghurt samples during cold storage. Different lowercase letters a-f show significance ($P < 0.05$).

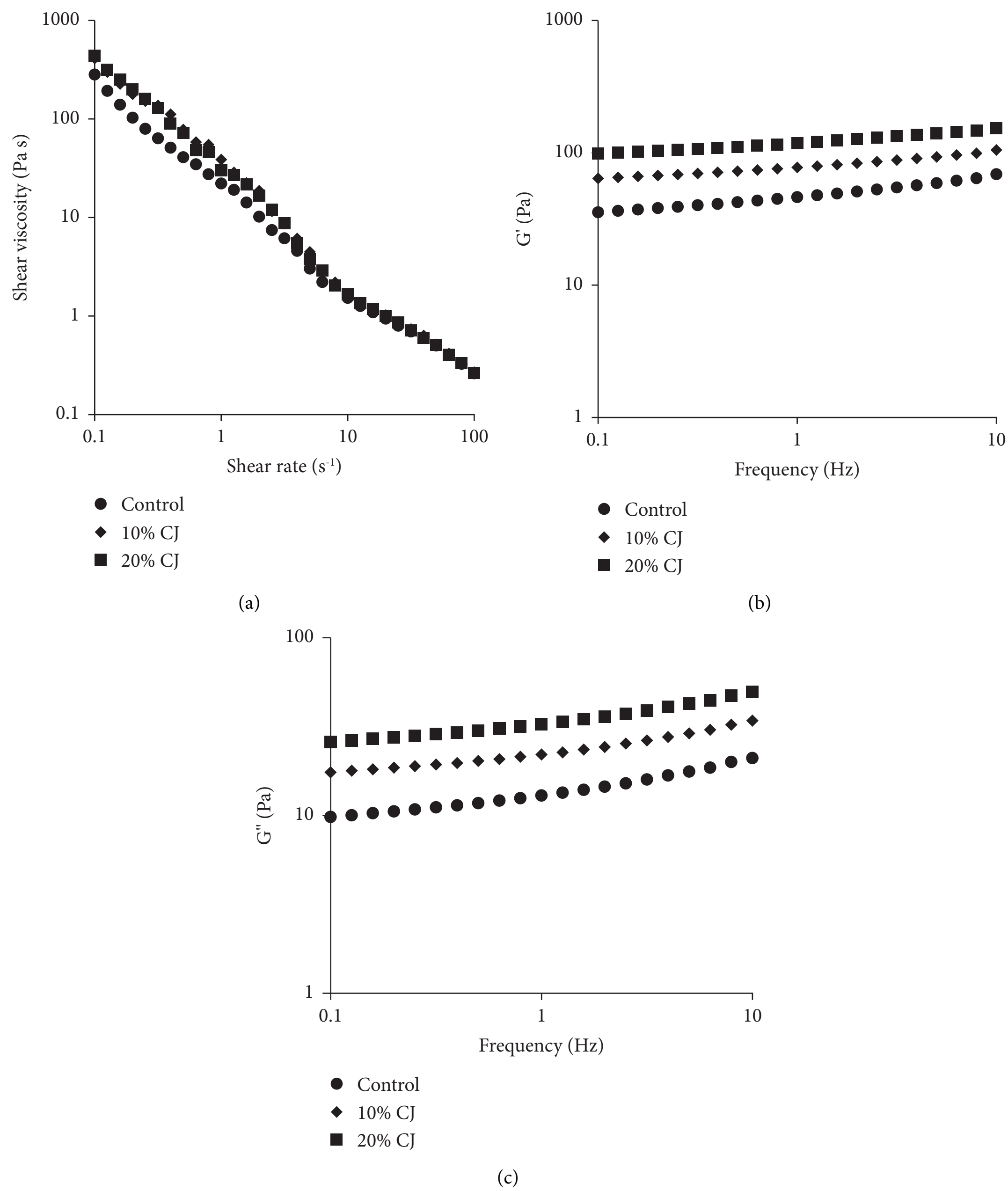

Figure 1: Apparent viscosity (a), elastic (G') (b), and viscous (G'') moduli (c) of the set-type yoghurt during storage (1 day).

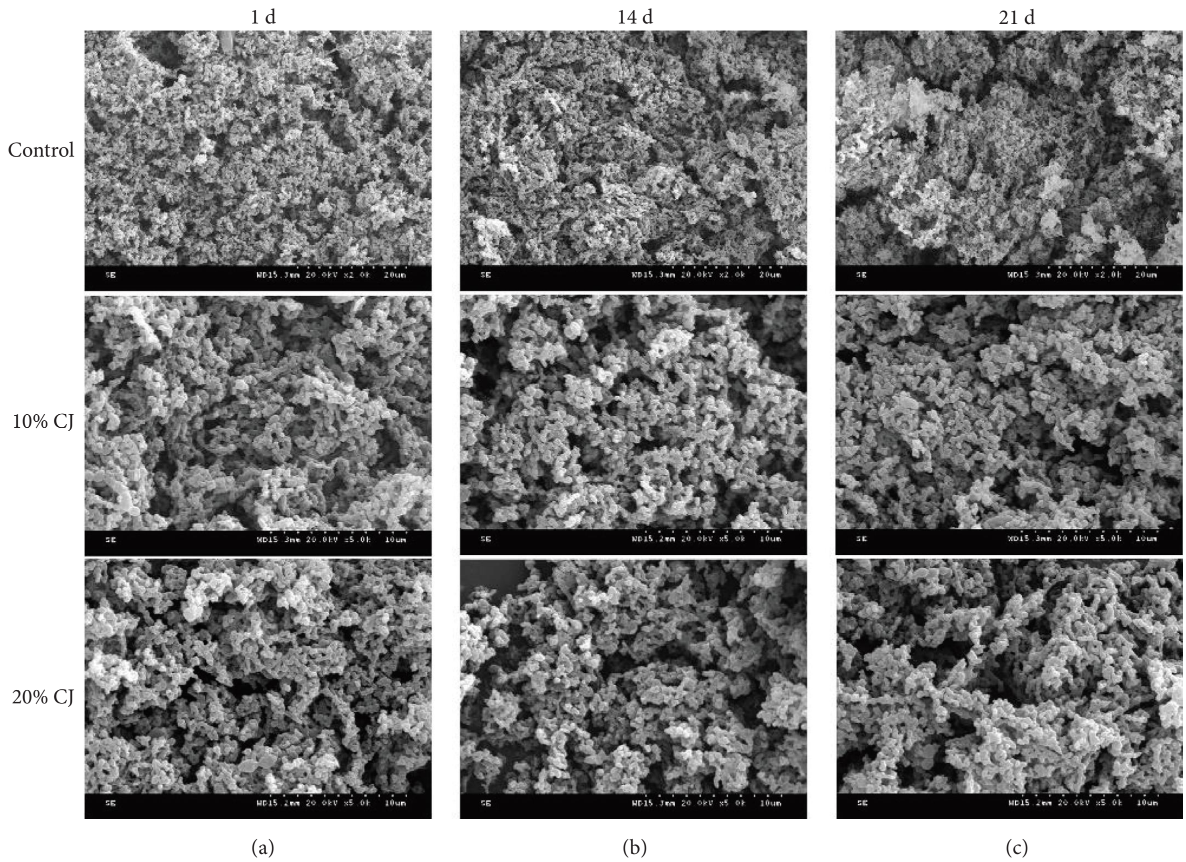

Figure 2: Scanning electron micrographs of yoghurts fermented containing (a) 0%, (b) 10%, and (c) 20% carrot juice (×2000).

influenced syneresis of yoghurt (Figure 3). In this study, all yoghurt samples demonstrated an increased syneresis extent ($P < 0.05$) with their storage period prolonged, showing the storage period resulted in enhanced yoghurt syneresis. However, from data comparation perspective, the results showed that the control sample at each time point made about the highest syneresis, whereas the yoghurt sample with 10% carrot had the lowest one. Thus, it is concluded that carrot juice had an obviously positive effect on yoghurt productions in reducing syneresis.

A previous study concluded that syneresis extent of yoghurt might be increased with extension of the storage period [49]. Under normal circumstances, dietary fibers or other macromolecules found in yoghurt can reduce syneresis extent of yoghurt; for example, the concentration of inulin in 30–150 g/kg could obviously reduce syneresis [50]. In another past study, yam mucilage increased viscosity of yoghurt and depressed yoghurt syneresis [51, 52]. In this study, carrot juice included polysaccharides that could enhance yoghurt viscosity. Therefore, the yoghurt samples with 10% carrot juice demonstrated less syneresis than the control samples. However, it is also observed that the yoghurt samples containing 20% carrot juice possessed higher syneresis than the control and the yoghurt with 10% carrot juice. It could be proposed that the higher viscosity obtained from a higher content of carrot juice addition might inhibit acid-caused aggregation of casein particles

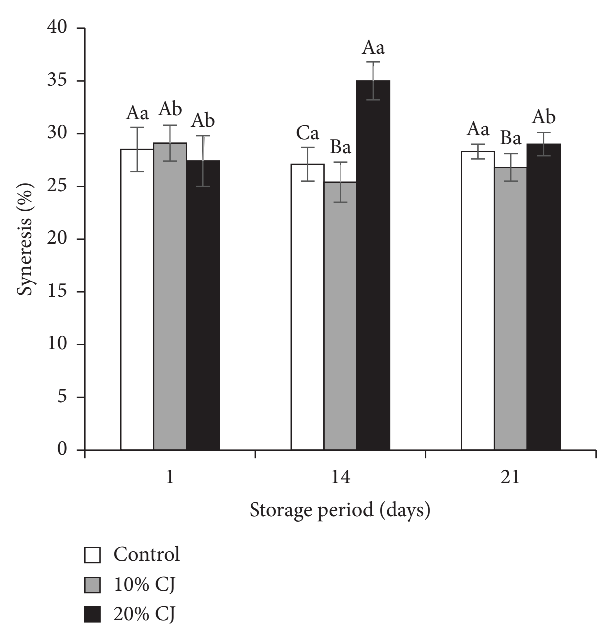

Figure 3: Syneresis values of carrot juice-fortified yoghurt during the cold storage period at 4°C. Values are showed as mean ± standard deviation. $^{a-c}$Means followed in the same row by different lowercase letters are significantly different for the same parameter ($P < 0.05$). $^{A-C}$Means followed in the same column by different capital letters are significantly different for the same parameter ($P < 0.05$).

during fermentation and have a negative effect on the microstructure of yoghurt. The yoghurt sample with 10% carrot juice might make about a finer microstructure and have less syneresis than the yoghurt sample containing 20% carrot juice.

Syneresis was found in yoghurt when gels spontaneously trend to shrink, leading to expelling liquid [53]. In the present study, there is a decreasing tendency of syneresis extent in the presence of 10% carrot juice compared to the control, whereas syneresis extent increased with the carrot juice level increased to 20% (Figure 3). Carrot juice is expected to include abundant pectin [6], which can dissolve during heating processing of CJ-milk mixture before fermentation as the ambient viscosity increase. Other soluble dietary fibers in carrot juice had similar influence. In addition, CJ contains prime various insoluble dietary fibre, which can interfere with gel structure continuity and result in an increase of syneresis extent, whereas the soluble composition would reduce syneresis extent by binding water and reinforcing the viscosity of gel continuous phase. Thereby, although increasing the CJ level to 20% provides very good water binding ability and viscosity increase, at the same time it also provides a higher vast of insoluble particles, which may interfere with the gel and enhance syneresis extent.

4. Conclusion

Addition of carrot juice enhanced the pH, reduced fermentation time (especially for the 10% carrot juice), and finally developed a firming and more homogeneous yoghurt gel during the storage period. The micrograph of microstructure was correlated with the rheological and texture analysis. Compared with other samples, the yoghurt with 10% carrot juice showed the firmest structure over 21-day storage time among the three levels of carrot juice determined. Thus, carrot juice has a potential advantage to be incorporated as a nature texturizer and stabilizer during yoghurt fermentation processing. In the future, we need to perform some sensory experiments to verify consumer recognition of supplemented yoghurt products, and colour or flavour regulations should also meet consumer needs.

Data Availability

The data that support the findings of this study are available on request from the corresponding author upon reasonable and uncommercial request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Zhi-Qin Pan performed the experiments. Li-Ying Bo conceived and designed the experiments. Li-Ying Bo and Chun-Li Song wrote the draft. Li-Ying Bo, Tian-ying Sun, Guo-Jun Du, Duo Zhang, and Zhi-Qiang Song finished the validation and analysis of the data. Chun-Li Song and Ren Jian supervised this programme.

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