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Improvement of Texture Properties and Flavor of Frozen Dough by Carrot (*Daucus carota*) Antifreeze Protein Supplementation

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The effects of concentrated carrot protein (CCP) containing 15.4% (w/w) carrot (*Daucus carota*) antifreeze protein on texture properties of frozen dough and volatile compounds of crumb were studied. CCP supplementation lowered the freezable water content of the dough, resulting in some beneficial effects including holding loaf volume steadily and making the dough softer and steadier during frozen storage. Furthermore, SPME-GC-MS analysis showed CCP supplementation did not give any negative influences on volatile compounds of crumb and gave a pleasant aroma felt like *Michelia alba DC* from *trans*-caryophyllene simultaneously. Combining our previous results that CCP supplementation improves the fermentation capacity of the frozen dough, CCP could be used as a beneficial additive for frozen dough processing.

KEYWORDS: Antifreeze protein; frozen dough; *Daucus carota*; freezable water content; texture profile analysis; SPME-GC-MS

INTRODUCTION

Antifreeze protein (AFP) can decrease the freezing point nonequilibriumly, referred to as thermal hysteresis activity, and retard recrystallization strongly (*1*). Even in the frozen state, AFPs inhibit the Ostwald ripening, particularly when ice approaches the melting point (*2*). Although only a few studies of AFPs on food processing are available, these studies prove the feasibility of AFPs in food processing (*3–5*).

The frozen dough technique has been of great interest since the 1960s, dealing with problems of short shelf life (*6*). This technique weakens the dough structure and decreases the retention capacity, although these defects can be avoided by using strong wheat flour or freeze-tolerant yeasts (*7, 8*). Furthermore, this technique prolongs fermentation times and deteriorates the texture of bread, although these drawbacks can be minimized by additives (*9–11*). Remarkably, additives change the texture properties or volatile compounds of the bread as well (*11*). As a result, some additives improve the bread quality, whereas others do not. Therefore, it is necessary to evaluate the effects of the supplementations or additives on the texture properties and volatile compounds of bread before processing (*12, 13*).

Our previous study proved that the concentrated carrot protein (CCP) containing 15.4% (w/w) carrot (*Daucus carota*) AFP (*Dc*AFP) improves the fermentation capacity of frozen dough (*14*). In this study, we evaluated the texture properties of dough and volatile compounds of crumb after CCP supplementation, confirming the feasibility of CCP in frozen dough processing.

MATERIALS AND METHODS

Preparation of CCP. CCP was prepared following the method we used earlier (*14*). Protein content was assayed with a modified Lowry protein assay kit (Pierce Biotechnology Inc., Rockford, IL) as described by the instructions. Bovine serum albumin (BSA) was used as a standard reference. Water, ash, and fat of the sample were determined according to the standard AACC methods (*15*).

Electrophoresis. SDS–PAGE was done according to Laemmli's discontinuous system with a slight modification (*14*). The sample was mixed at 1:1 (v/v) ratio with SDS–PAGE sample buffer (0.125 M Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 10% β-mercaptoethanol) and boiled for 5 min. The boiled sample (10 μL) was loaded onto the gel made of 4% stacking and 12.5% separating gels, following by being subjected to a constant current of 15 mA per gel using a Mini-Protean III cell apparatus (Bio-Rad Laboratories, Mississauga, Ontario, Canada). Then the gels were stained with 0.1% Coomassie Brilliant Blue R-250 in 50% methanol and 7% acetic acid and destained with 7% acetic acid. The molecular mass was estimated by the sample's relative mobility in gel compared to those of the low molecular mass markers (Amersham Pharmacia Biotech, Sweden).

Preparation of Stock Dough. A stock dough was prepared according to the following formula: 1000 g of wheat flour, 20 g of

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Table 1. Composition of CCP Powder

composition	protein	water	fat	ash
CCP powder (g/100 g)	84.2 ± 5.4	9.5 ± 0.3	–[a]	0.4 ± 0.1

[a] Not detected.

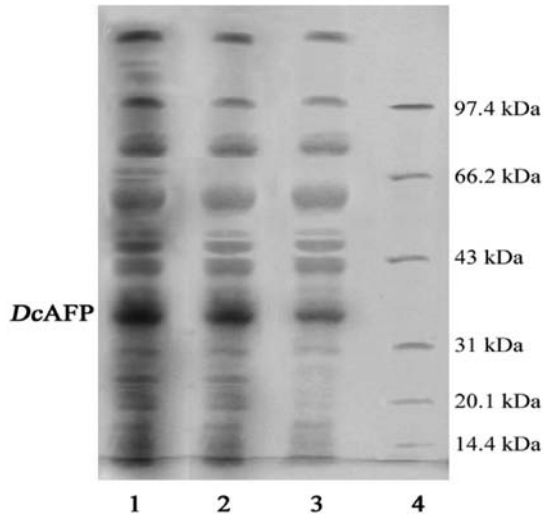

Figure 1. SDS–PAGE gel of CCP. Lanes 1–3 are CCP powder. Specifically, aliquots of 150, 100, and 50 μg of CCP powder were loaded in lanes 1, 2, and 3, respectively. *Dc*AFP is 18.41%, 19.83%, and 16.68% gray of total CCP in lanes 1, 2, and 3, respectively. Lane 4 is the low molecular mass marker including rabbit phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), rabbit actin (43 kDa), bovine carbonic anhydrase (31 kDa), trypsin inhibitor (20.1 kDa), and hen egg white lysozyme (14.4 kDa).

instant yeast, 40 g of sucrose, 15 g of NaCl, 620 g of water, 50 g of butter, and 6.2 g of protein [including BSA, soy protein isolated (SPI), or CCP, respectively]. The control had the same formula except for 6.2 g of protein. Yeast, sucrose, and protein were stirred in water before being added to the flour. Other ingredients were added in solid form when the dough was nearly formed. The simplicity of this formula allowed a clear observation of changes during the processing of frozen dough. The resulting dough (120 g) was molded, covered with polymer film, and stored at −30 °C immediately. The molded frozen dough was thawed in a fermentation cabinet at 38 °C and 90% relative humidity for 160–180 min. Then the molded dough was baked in an oven (XK01; Shanghai Bud Food Machine Co. Ltd., Shanghai, China) with 180 °C top temperature and 210 °C bottom temperature for 20 min.

Sensory Evaluation. A panel of nine members was selected randomly from local staff members. They were trained and instructed to score total volume (35 points), texture structure (25 points), flatness (10 points), flexibility and plasticity (10 points), loaf shape and crust texture (5 points), crust color (5 points), crumb color (5 points), and mouth feel (5 points) of bread according to the criterion of the National Bread Sensory Evaluation Standard (National Standard: GB14611-93, China). The National Bread Sensory Evaluation Standard is used, because it is more general and rigorous than methods described earlier (*13*, *16*). The national standard gave the panel a detailed guideline to score the product. For example, the total score of the mouth feel is 5 points and is classed into five grades. (1) 4–5 points: The bread has the flavor from the Maillard reaction and the yeast fermentation and tastes chastely without any moldiness. Half of a point is subtracted if the bread tastes rough. (2) 3–4 points: The bread has the taste of the sugar and salt without baking aroma and peculiar smell. Half of a point is subtracted if the bread is cracked or drops its crumb easily. (3) 2–3 points: The bread tastes unchastely with a slight sourness or peculiar smell. (4) 1–2 points: The bread tastes unchastely with a slight peculiar smell and is cracked easily. (5) 0–1 points: The bread tastes moldy and drops its crumb easily. The entire criterion can be found in the National Bread Sensory Evaluation Standard (GB14611-93, China).

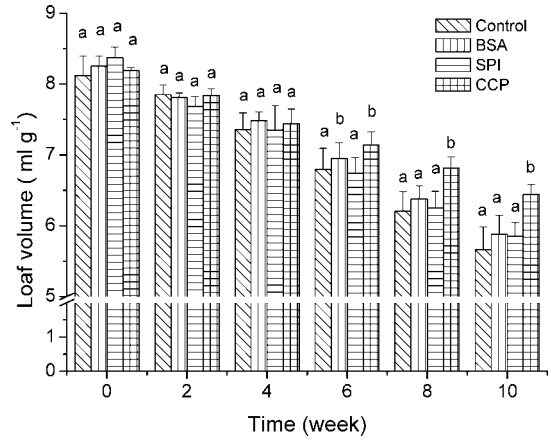

Figure 2. Effect of frozen storage on the loaf volume. Data are the means ± standard deviation ($n \geq 3$). Means with different letters in the same time represent a significant difference ($P < 0.05$).

A sample was sliced into a l cm thick piece and evaluated 3 h after baking. From each sample group, one slice of sample was offered to every panelist at the same time in an open area without special lighting. Water was provided for rinsing.

Determination of Loaf Volume. Loaf volume was measured by rapeseed displacement, according to AACC method (*15*). The variational rate (ϵ) evaluates the changes of loaf volume between the bread from the fresh dough and the bread from the dough with frozen storage, calculated by eq 1:

$$\epsilon = \frac{V_S - V_E}{V_S} \times 100\% \quad (1)$$

where V_S is the bread from the fresh dough (mL/g) and V_E is the bread from the dough with frozen storage (mL/g).

Determination of Freezable Water Content by the Differential Scanning Calorimeter Method. The freezable water content (Δ) of a sample was determined by the differential scanning calorimeter (DSC; Diamond DSC-7; Perkin-Elmer Pyris, Boston, MA) method (*17–19*). After being stored at −30 °C for 48 h, the water of a sample (W_A) was determined as prescribed by the AACC method (*15*). The content of the freezable water of a sample (W_C) was determined by the following method. First, aliquots of 10 mg of a sample were taken from the center of the stock dough, flatted in an aluminum pan, and stored at −30 °C for 48 h. Then the sealed pan was quickly moved to a prefrozen stove of DSC, holding at −30 °C for 5 min, and then raising to 15 °C at rate of 3 °C/min. The endothermic enthalpy (ΔH_W) of a sample was recorded (statistic analysis done by Pyris Software for Windows Version 3.80). The W_C was calculated by the endothermic enthalpy of water ($\Delta_{fus}H_m = 333.3J/g$). The freezable water content of a sample (W_C) is calculated by eq 2:

$$W_C = \frac{\Delta H_W}{\Delta_{fus}H_m m} \quad (2)$$

where ΔH_W is the endothermic enthalpy (J) of the sample, $\Delta_{fus}H_m = 333.3J/g$, and m is sample weight (g).

The freezable water content (Δ) is calculated by eq 3:

$$\Delta = \frac{W_C}{W_A} \times 100\% \quad (3)$$

where W_C is the content of the freezable water of the sample and W_A is the content of the water of the sample.

Texture Profile Analysis of Bread and Dough. Texture profile analysis of bread simulates the chewing movements and has been accepted universally (*20*). The analysis was done by a texturometer equipped with a 25 mm diameter aluminum cylinder (Stable Microsystems TA-XT2i, Scarsdale, NY). The results include hardness,

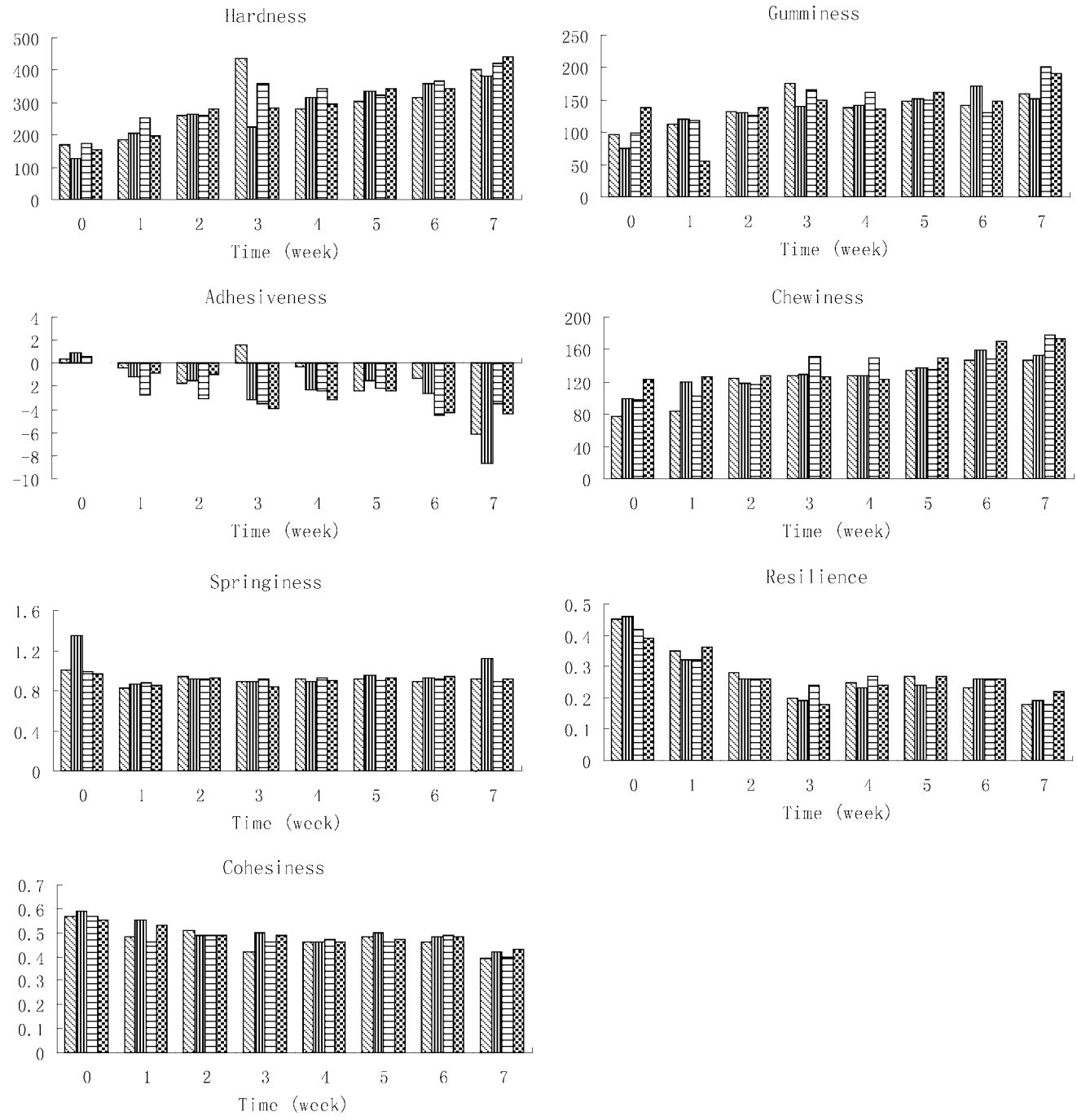

Figure 3. Effect of frozen storage on the texture profile analysis. Key: control, cross-hatched bar; BSA, vertically striped bar; SPI, horizontally striped bar; CCP, dotted bar.

fracturability, adhesiveness, springiness, chewiness, gumminess, cohesiveness, and resilience, as defined by Fiszman et al. (*20*). For each experiment, a sample was sliced into a l cm thick piece, and two slices were placed on the control desk. The compression test was set as follows: pretest speed, 3.0 mm/s; test speed, 1 mm/s; posttest speed, 5.0 mm/s; distance, 50%; time, 5.00 s.

The hardness of the frozen sample was measured by a texturometer during the freezing process (Stable Microsystems TA-XT2i, Scarsdale, NY) equipped with a 25 mm diameter aluminum cylinder. The sample (120 g) was stored in an aluminum column (Φ 60 mm × 50 mm) at −80 °C for 0, 20, and 40 min, respectively. The surface center of the sample was compressed at the speed of 3.0 mm/s to 80% distance. The peak force was denoted as hardness.

Determination of Crumb Volatile Compounds by SPME-GC-MS Analysis. The volatile compounds of crumb were determined by SPME-GC-MS analysis. The volatile compounds were absorbed by the SPME sampling method using a 75 μm CAR/PDMS absorbing pin (*21*, *22*). Specifically, volatile compounds of 3 g of a sample were absorbed in a 20 mL glass vial at 50 °C for 35 min, followed by being desorbed in the injector port of a gas chromatograph at 250 °C. Separation was done on a gas chromatograph column (PEG 20 M; DB-WAX, 30 m × 0.25 mm × 0.25 μm; Agilent Technologies, Inc., CA) with helium at 0.80 mL/min. The oven temperature was programmed at 40 °C for the first 3 min and then raised to 60 °C (5.0 °C/min), 130 °C (6.0 °C/min), and 230 °C (10.0 °C/min). Then a Finnigan Trace MS (70 eV ionization energy, *m*/*z* 29–400 mass range) was used for the analysis of the total ion chromatograph. Identification of compounds was based on matching with commercial mass spectra NBS/WILEY libraries or comparing Kovats retention indices (*I*), which was calculated by C_5–C_{22} alkanes, or comparing the retention time (RT) of pure references.

In order to evaluate the effect of CCP supplementation on crumb volatile compounds, a new group denoted as the carrot group was evaluated. The sample of the carrot group was a solution of 500 mg of CCP powder dissolved in 2.5 mL of 50 mM Tris-HCl buffer (pH 7.4).

Table 2. Bread Sensory Evaluation Ranked by Nine Panelists According to the National Bread Sensory Evaluation Standard (China)[a]

item	control	BSA	SPI	CCP
total volume (35 points)	31.4 ± 1.4^a	32.3 ± 0.8^a	32.3 ± 1.3^a	33.0 ± 1.6^b
texture structure (25 points)	20.9 ± 1.5^a	21.9 ± 1.4^a	21.6 ± 1.4^a	21.9 ± 1.5^a
flatness (10 points)	5.7 ± 1.2^a	6.0 ± 1.2^b	6.4 ± 1.1^c	6.2 ± 1.3^b
flexibility and plasticity (10 points)	8.8 ± 0.8^a	8.4 ± 1.0^b	8.9 ± 0.7^a	8.9 ± 0.7^a
bread sharp and crust texture (5 points)	4.1 ± 0.7^a	4.5 ± 0.5^b	4.4 ± 0.5^b	4.4 ± 0.7^b
crust color (5 points)	3.1 ± 0.7^a	3.8 ± 0.6^b	3.5 ± 0.7^c	3.6 ± 0.7^c
crumb color (5 points)	3.4 ± 0.8^a	3.4 ± 0.5^a	3.8 ± 0.8^b	3.8 ± 0.7^b
mouth feel (5 points)	3.5 ± 0.5^a	3.7 ± 0.7^b	3.8 ± 0.7^c	3.9 ± 0.7^c
sum	80.90	83.95	84.70	83.70

[a] Data in the same row with different letters as superscripts are significantly different ($P < 0.05$).

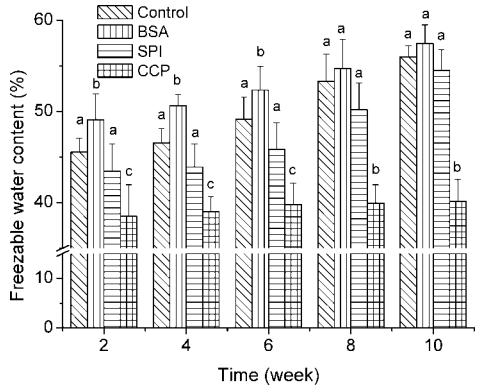

Figure 4. Changes of freezable water content during frozen storage. Data are the means ± standard deviation ($n \geq 3$). Means with different letters in the same time represent a significant difference ($P < 0.05$).

Statistical Analysis. All data were expressed as the mean value ± standard deviation ($n \geq 3$). All statistical analyses were done with the Super ANOVA (version 1.11; Abacus Concepts Inc., Berkeley, CA). One-way ANOVA and multiple comparisons (Fisher's least significant difference test) were used to evaluate the significant differences of data at a criterion of $P < 0.05$.

RESULTS AND DISCUSSION

Composition and Molecular Mass Distribution of CCP. The protein content of CCP powder is 84.2% (w/w) (**Table 1**). Meanwhile, the composition of CCP powder was measured to confirm the existence of *Dc*AFP by SDS–PAGE analysis. Results showed that *Dc*AFP was 18.41%, 19.83%, and 16.68% gray of total CCP in the SDS–PAGE gel (statistic analysis done by Glyko Bandscan version 5.0) (**Figure 1**). *Dc*AFP was 18.3 ± 1.58% gray of carrot protein when carrot protein was 84.2% (w/w) of CCP. Therefore, *Dc*AFP was 15.4% (w/w) of CCP. Meanwhile, CCP was 1 g/100 mL of water in the dough as shown in the formula of frozen dough. Combined with the dough formula, *Dc*AFP was 1.54 mg/mL water in the dough. Our *Dc*AFP includes a single band at 36 kDa, same as the known *Dc*AFP (*14*, *23*). Therefore, *Dc*AFP was 1.54 mg/mL of water in the dough.

Evaluation of Sensory and Texture Quality of Bread. In order to estimate the effect of CCP supplementation on bread quality, the bread quality was evaluated by loaf volume, texture profile analysis, and the National Bread Sensory Evaluation Standard (China). The loaf volume decreased with the frozen storage (**Figure 2**), because the ice crystals pierce into the yeast and decrease the yeast survival during frozen storage, lowering the fermentation capacity (*14*). The loaf volume of the CCP group showed significant difference with that of the control after 6 weeks of storage. Specifically, the loaf volume of the control, BSA, SPI, and CCP groups was from 8.12 to 5.66 mL/g, from 8.25 to 5.88 mL/g, from 8.37 to 5.85 mL/g, and from 8.19 to 6.44 mL/g. The loaf volume of the CCP group was the largest among the tested groups after 10 weeks of frozen storage. The ϵ of the control, BSA, SPI, and CCP groups was 30.30%,

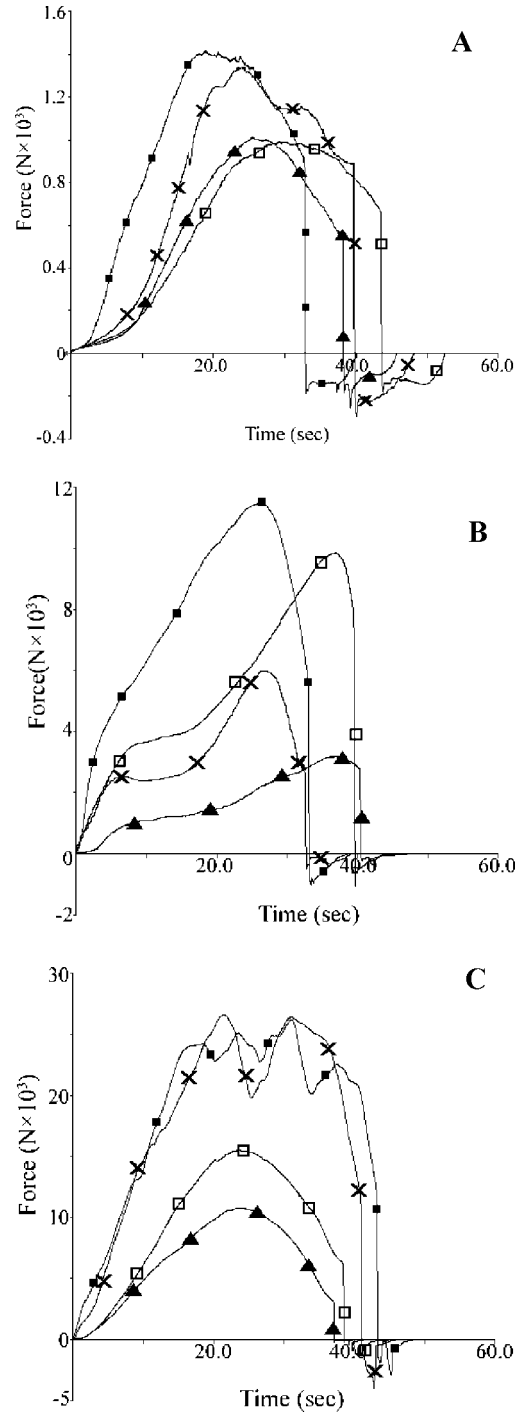

Figure 5. Effect of frozen storage on dough texture property. (A) Fresh dough. (B) Frozen for 20 min at −80 °C. (C) Frozen for 40 min at −80 °C. Key: control, ×; BSA, ■; SPI, □; CCP, ▲.

Table 3. Volatile Compounds of Crumb Evaluated by SPME-GC-MS

				carrot		control		BSA		SPI		CCP	
no.[a]	compound[b]	ID[c]	*I*[d]	RT[e]	area (%)[f]	RT	area (%)	RT	area (%)	RT	area (%)	RT	area (%)
1	ethanol	1, 2, 3	910	–[g]	–	5.07	26.35	5.17	17.82	5.07	23.86	5.10	22.13
2	3-methylbutanal	1, 2	929	–	–	–	–	–	–	–	–	6.24	0.29
3	toluene	1, 2, 3	985	–	–	–	–	–	–	–	–	8.00	0.85
4	hexanal	1, 2, 3	1104	–	–	9.29	2.73	9.33	1.93	9.29	4.60	9.31	2.64
5	1-dodecene	1, 2, 3	1118	–	–	–	–	–	–	–	–	9.48	0.81
6	8-methyl-1-undecene	1, 2	1124	–	–	–	–	–	–	–	–	9.6	0.57
7	2-methyl-1-propanol	1	1129	–	–	–	–	–	–	–	–	9.95	1.53
8	ethylbenzene	1, 2, 3	1161	–	–	–	–	–	–	–	–	10.54	7.17
9	heptanal	1, 2, 3	1174	–	–	12.40	1.20	12.43	0.80	12.30	1.21	11.42	0.98
10	3,5-dimethyloctane	1, 3	1195	–	–	–	–	–	–	–	–	12.53	0.63
11	3-methyl-1-butanol	1, 2, 3	1205	–	–	–	–	13.11	19.10	13.31	18.36	13.10	12.73
12	hexanoic acid ethyl ester	1, 2	1229	–	–	13.74	0.80	13.84	0.77	13.58	1.58	13.83	1.30
13	1-pentanol	1, 2, 3	1255	–	–	14.30	1.23	14.25	0.64	14.38	1.27	14.24	1.17
14	3-hydroxy-2-butanone	1, 2, 3	1266	–	–	15.33	1.69	15.34	1.69	–	–	15.32	2.07
15	2-hydroxypropanoic acid ethyl ester	1, 2	1348	–	–	16.72	0.43	16.73	0.49	–	–	16.72	0.24
16	1-hexanol	1, 2, 3	1360	–	–	16.8	7.47	16.84	7.26	16.88	6.66	16.84	6.20
17	nonanal	1, 2, 3	1385	17.88	0.03	17.88	1.99	17.89	1.09	17.77	1.09	17.88	1.05
18	octanoic acid ethyl ester	1	1429	18.77	0.16	–	–	–	–	18.69	7.69	18.78	7.46
19	1-octen-3-ol	1, 2	1297	–	–	19.09	0.41	19.09	0.24	19.07	0.33	19.09	0.40
20	1-heptanol	1, 2, 3	1310	–	–	19.19	0.75	19.19	0.51	19.19	0.61	19.19	0.93
21	furfural	1, 2, 3	1483	–	–	19.59	0.73	19.59	0.15	19.58	0.58	19.59	0.79
22	acetic acid	1, 2, 3	1450	–	–	–	–	–	–	–	–	19.70	0.31
23	2-ethyl-1-hexanol	1, 3	1489	–	–	–	–	19.89	0.45	–	–	19.89	2.55
24	benzaldehyde	1, 2, 3	1569	–	–	20.80	1.28	20.75	2.05	20.72	1.25	20.75	3.20
25	2,3-butanediol	1, 2, 3	1581	–	–	20.89	2.58	20.90	1.14	20.92	2.21	20.89	1.85
26	1-octanol	1, 2, 3	1608	21.14	0.06	21.10	0.46	21.11	0.31	21.10	0.29	21.12	0.68
27	2-methylpropanoic acid	1, 2, 3	1621	–	–	21.48	4.62	21.50	4.04	21.52	3.75	21.49	3.65
28	*trans*-caryophyllene	1, 2	1638	21.99	23.85	–	–	–	–	–	–	21.89	1.30
29	butanoic acid	1, 2, 3	1634	–	–	22.49	0.83	22.51	0.71	–	–	22.50	0.77
30	1-nonanol	1, 2, 3	1695	22.72	0.05	22.71	0.36	22.71	0.45	–	–	22.72	0.32
31	3-methylbutanoic acid	1, 2, 3	1703	–	–	23.03	3.22	23.04	3.82	23.06	3.56	23.04	2.24
32	3-nonen-1-ol	1, 3	1712	–	–	23.09	1.05	–	–	–	–	23.09	0.75
33	decanoic acid ethyl ester	1, 2, 3	1728	–	–	23.42	0.20	23.42	0.42	23.42	0.10	23.42	0.63
34	dodecanoic acid ethyl ester	1, 2	1818	–	–	–	–	25.14	0.13	25.13	0.46	25.14	0.42
35	hexanoic acid	1, 2, 3	1850	–	–	25.32	1.41	25.32	2.03	25.36	1.97	25.32	1.68
36	benzyl alcohol	1	1862	–	–	25.69	0.48	25.69	0.56	25.70	0.46	25.69	0.46
37	phenylethyl alcohol	1, 2	1873	–	–	26.11	5.53	26.11	5.34	26.11	3.83	26.11	3.35
38	phenol	1, 2, 3	2004	–	–	27.16	0.04	27.16	0.06	27.16	0.04	27.16	0.05
39	octanoic acid	1, 2, 3	1996	–	–	27.68	3.25	27.65	3.03	27.7	1.09	27.66	0.95
40	decanoic acid	1, 2, 3	2361	–	–	29.76	0.03	29.76	0.05	29.81	0.04	29.76	0.05
total area (%)					24.15		71.12		77.08		86.89		97.16

[a] Peak number. [b] Compound determined. [c] Identified by (1) mass spectra match, (2) Kovats retention indices, and (3) authentic compound. [d] Kovats retention indices calculated for 20 M PEG, 30 m × 0.25 mm × 0.25 μm columns, in GC-MS using C_5–C_{22} alkanes. [e] Retention time (minutes). [f] Area content. [g] Not detected.

28.73%, 30.10%, and 21.37%, respectively. The ϵ of the CCP group was the smallest one of the four groups, proving that the loaf volume of the CCP group was more steady than that of other groups. Therefore, the CCP group had the biggest loaf volume and strongest ability in holding loaf volume among the tested samples during frozen storage.

Texture profile analysis shows the effect of frozen storage on texture profile in 7 weeks (**Figure 3**). The fracturability was not present, because it was not detected in most of the tests. Meanwhile, the results of hardness, adhesiveness, springiness, chewiness, gumminess, cohesiveness, and resilience of the CCP group did not show significant differences with those of the control. Moreover, the trends of hardness, adhesiveness, springiness, chewiness, gumminess, cohesiveness, and resilience of the CCP group were similar to those of the control statistically. Therefore, the results of texture profile analysis of the CCP group were similar to those of other groups during frozen storage.

The results of bread sensory quality evaluation ranked by nine panelists according to the National Bread Sensory Evaluation Standard (China) are shown in **Table 2**. The score of the total volume of the CCP group showed a significant difference with that of the control, mainly due to the fact that the loaf volume of the CCP group was larger than that of the control. The crust color of the CCP group was deeper than that of the control because of the Maillard reaction induced by CCP supplementation. The crumb color of the CCP group was whiter than that of the control and showed a significant difference with that of the control. Likewise, the mouth feel of the CCP group was softer and smoother than that of the control. Other items of the CCP group were similar to those of the control statistically. The score of the CCP group was 83.70 points, similar to the mean score of the total scores of the tested groups that was 83.31 ± 1.66 points.

In summary, CCP supplementation brought a stronger ability in holding loaf volume and did not give any negative influence on the texture profile analysis and bread sensory quality.

Effect of Frozen Storage on Freezable Water Content. The freezable water content (Δ) is an important factor to control the quality of frozen dough (*9*, *24*). The Δ of the CCP group was steady from 38.50% to 40.12% in 10 weeks of storage, showing a significant difference with that of other groups

(**Figure 4**). Furthermore, the curve slope of Δ of the CCP group was also lower than that of other groups. On the other hand, the difference of the Δ was from the BSA, SPI, and CCP supplementation specifically, because all other factors that influenced the Δ were fixed. It is CCP supplementation that lowered the Δ of the CCP group. Therefore, the effect of frozen storage on freezable water content was smaller in the CCP group than that in the control. Meanwhile, our results were lower than the mean value (67%) reported earlier (*6*), probably due to differences in raw material or bread formula mainly.

AFPs can absorb free water around them with the consequence to restrict a free movement of water (*25*). Therefore, unfreezable water in the CCP group was limited by being absorbed to *Dc*AFP. In contrast, the unfreezable water in other groups worked as the free water in the dough. Remarkably, the free water can lead to a cycle of Ostwald ripening or recrystallizing during the frozen storage, even resulting in the subsequent damage to the gluten matrix and death of yeast (*14*, *26*). Therefore, the final bread will be inferior in both texture properties and loaf volume when the free water content is high, consistent with our results.

Changes of Dough Hardness during the Freezing Process. The hardness changes of the dough were measured during the freezing process (**Figure 5**). The force curve of fresh dough is shown during the compressing process (**Figure 5A**). The hardness of the control, BSA, SPI, and CCP groups was 13364.85, 13246.27, 8590.68, and 8795.99 N, respectively. The hardness curve of dough frozen for 20 min is shown in **Figure 5 B**. The hardness of the control, BSA, SPI, and CCP groups was 59762.85, 113282.41, 95435.24, and 28162.75 N, respectively. The hardness curve of dough frozen for 40 min is shown in **Figure 5 C**. The hardness of the control, BSA, SPI, and CCP groups was 291864.48, 218079.498, 130034.04, and 111916.88 N, respectively. The hardness of the CCP group was smaller than that of the other groups when frozen for 20 and 40 min, proving that the dough with CCP supplementation was softer than that of the other groups when frozen. On the other hand, the hardness of the CCP group frozen for 40 min was 12.72 times to the hardness of the corresponding fresh dough; in contrast, that of the control, BSA, and SPI groups was 21.84, 16.46, and 15.14 times to the hardness of the corresponding fresh dough. The multiple intensity of the CCP group was smaller than that of other groups. The hardness change of the CCP group was smaller than that of the other groups during frozen storage. Therefore, the hardness of the CCP group was softer and steadier than that of the other groups.

The hardness changes referred to mainly resulted from the lower freezable water content (*6*, *11*, *12*). The lower the freezable water content, the lower the ice crystal content. The ice crystal content of the CCP group was smaller than that of the other groups because of its low freezable water content. Remarkably, the sharp ice crystals will pierce into the yeast and decrease the yeast survival (*27*). So the fermentation capacity is improved, and the texture property is better when the freezable water content is low (*14*, *19*). The damage of the sharp ice crystals to the dough matrix was also minimized in the CCP group. Therefore, the texture property was improved by CCP supplementation.

Evaluation of Volatile Compounds by SPME-GC-MS Analysis. The volatile compounds of the carrot, control, BSA, SPI, and CCP groups are summarized in **Table 3**. Specifically, all of the determined volatile compounds of the CCP group were listed. However, only the compounds that were same as that of the CCP group were listed for the carrot, control, BSA, and SPI groups. The carrot group was analyzed as the position control to evaluate the differences of volatile compounds of CCP in water or in bread.

The carrot and CCP groups had five mutual volatile compounds, being 24.15% area and 9.81% area, respectively. The mutual *trans*-caryophyllene, which was the main pleasant aroma in *Michelia alba DC* (*28*–*30*), was 23.85% area and 1.30% area in the carrot and CCP groups, respectively. However, *trans*-caryophyllene was not detected in the other groups. Furthermore, *trans*-caryophyllene was a nature compound and was not from the Maillard reaction. Therefore, CCP supplementation brought a new pleasant aroma, *trans*-caryophyllene, to crumb.

Furthermore, the content of mutual compounds was 71.12% area and 60.54% area in the control and CCP groups, respectively, 77.08% area and 75.79% area in the BSA and CCP groups, respectively, and 86.89% area and 77.00% area in the SPI and CCP groups, respectively. More than 60% volatile compounds of the CCP group were the same to those of the control and the published literature (*31*). Therefore, CCP supplementation did not give a negative influence on volatile compounds of crumb and brought a pleasant aroma felt as from *Michelia alba DC* by *trans*-caryophyllene simultaneously.

ABBREVIATIONS USED

AFP, antifreeze protein; BSA, bovine serum albumin; CCP, concentrated carrot protein; *Dc*AFP, carrot (*Daucus carota*) antifreeze protein; DSC, differential scanning calorimetric; SPI, soy protein isolated; SPME-GC-MS, solid-phase microextraction–gas chromatograph–mass spectra.

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