Effect of freeze-thaw treatment on the quality and flavor of Nang

ZOU Shuping¹,²,a, QIU Ju³,b*, MENG Xintao¹,²,c, MENG Yina¹,²,d, MA Yan¹,²,e, XU Mingqiang¹,²,f, ZHANG Qian¹,²,g

¹Research Institute of Farm Products Storage and Processing, Xinjiang Academy of Agricultural Sciences, Urumqi, China
²Research Center of Main Agricultural Products Processing Engineering in Xinjiang, Urumqi, China
³Institute of Food and Nutrition Development, Ministry of Agriculture and Rural Affairs, Beijing, China
aemail: 674111327@cau.edu.cn, cemail: 455894201@qq.com, demail: 87947088@qq.com, eemail: 282568949@qq.com, femail: 372661331@qq.com, gemail: xjnkyzhq@qq.com,
*Corresponding author: bemail: qiuju@caas.cn.

Abstract: Freezing processing has been used widely to improve the sensory quality and solve the problems induced by fungi. In this study, the effects of frozen temperature (-20, -30, -40, -50, -60°C), frozen time (30, 40, 50, 60, 70 min), thawing temperature (25, 30, 35, 40, 45°C) and thawing time (10, 15, 20, 25, 30 min) on sensory quality, texture characteristics and flavor of Nang were studied. The results showed that the order of the factors affecting the sensory quality of Nang was frozen temperature > thawing time > frozen time > thawing temperature. The optimum processing conditions were to freeze at -50°C for 50 min and thaw at 40°C for 20 min. The correlation analysis between sensory quality and texture profile analysis (TPA) showed that the highest or the lowest TPA values of the core and edge of Nang did not correspond to the best sensory quality. Frozen treatment affected the characteristic flavor of Nang obviously. There were 15 of flavor substances with a significant increase or decrease among the 41 characteristic ones identified. This study is of great significance for the production and promotion of traditional staple food in Xinjiang.

1. Introduction
Since its market introduction in the 1960s, the frozen food industry has developed rapidly, with sales volume in some countries now accounting for 50% of the total commercial food sector[1-2]. In China, the growth of this industry has been spurred by continuous improvements in cold chain technology, the popularization of domestic-use microwave and baking ovens, as well as the ever-accelerating pace of life[3]. Furthermore, the quality and taste of quick-frozen food have also continuously improved, leading to increased popularity among growing numbers of consumers, who value such products only because they save time, space and cost but also for their freshness and rich flavors[4-6]. In the freezing process, the freezing rate, freezing storage temperature, thawing temperature and thawing period are the key factors affecting the quality of frozen products [7-9], and several research studies have investigated the impacts thereof on various foods. In terms of freezing conditions, for example, Yi et
al.\textsuperscript{[10]} found that the yeast survival rate was higher and the volume of the dough was larger when the freezing rate of dough was 19-41°C/h and the freezing storage temperature was -20--15°C. Similarly, Akbraian et al.\textsuperscript{[11]} studied frozen dough and found that higher freezing temperatures significantly decrease the cohesive and elastic qualities of the bread. In the study of spaghetti, it was found that the lower the freezing temperature, the more similar the texture and sensory properties of the product were to fresh spaghetti \textsuperscript{[12]}. Leray et al.\textsuperscript{[13]} found that the changes in rheological properties of the frozen dough stored at -30°C were much more obvious than the dough stored at -18°C, while the quality of dough stored at -18°C was better. In studies of the thawing process, Zhang et al.\textsuperscript{[14]} thawed frozen dough at 25°C and 30°C respectively and found that the effects on product quality were significantly different. Recent studies have mainly focused on the quality changes in products or semi-finished products\textsuperscript{[20]}, such as steamed buns\textsuperscript{[15]}, dumplings\textsuperscript{[16]}, noodles\textsuperscript{[17-18]} and pizza\textsuperscript{[10]}, however little has been reported about the effects of the freeze-thaw process on the quality of ‘Nang’, one of Northwest China’s traditional baked products.

As a staple flour-based food of the Uyghur people in Xinjiang province\textsuperscript{[21]}, Nang is nutritious and easy to carry, making it highly popular with locals as well as those in other regions\textsuperscript{[22]}. Modern food processing has refined the production of Nang, enabling the industrialization of these crispy, delicious and diversified baked pancake-style breads\textsuperscript{[23]}. Hence, the development of a Nang frozen dough both enriches the diversity of Nang products and meets the requirements of consumers for fast or convenient food. The effects of freeze-thaw treatment on the sensory and flavor of Nang are, thus, investigated in this study so as to provide theoretical support for the development and application of Nang frozen dough.

2. Materials and methods

2.1. Materials
The flour used in this study was of Special First Class and was obtained from Xinjiang Tianshan Flour Group Co., Ltd.; the yeast was from Angel Yeast Co., Ltd.; salt was from Urumqi Salt Industry Co., Ltd.; sugar was from Urumqi Quandeli Industry and Trade Co., Ltd.; and edible oil (corn oil) was from COFCO Fulinmen Food Marketing Co., Ltd.

2.2. Instruments and equipment
Instruments and equipment employed for analysis in this study included a FlavourSpec gas-phase ion migration spectrometer (G.A.S., Germany) and a TMS-Pro texture analyzer (FTC, USA). Dough fermentation was carried out in a controlled fermentation cabinet (JXFD fermenting box, Dongfu Jiuheng Instrument Technology Co., Ltd., Beijing, China), and it was then mixed in a KVL Dough Maker (JVCKENWOOD Electronics Trading (Shanghai) Co., Ltd.). Chilling and freezing were conducted in a DW Low Temperature Refrigerator and DW Ultra-low Temperature Freezer, respectively (Haier Biomedical, Qingdao, China). Baking was done in a SM2-901C Oven (Wuxi Xinmai Machinery Co., Ltd., China). Weighing was conducted using an ML204 Analytical Balance (Mettler Toledo International) and a TD10KA electronic weighing scale (Nanjing Suci Measuring Instrument Co., Ltd.).

2.3. Methods

2.3.1. Preparation of raw Nang dough
The preparation of Nang begins with flour, kneading, fermentation, reshaping, second fermentation, finalization of the design, patterning, and coating in order to create the raw dough. In this study, first, the flour was weighed, then an appropriate amount of water and auxiliary materials were added and the mixture was kneaded to make a smooth dough, which was placed in a fermenting box at 38°C and humidity 80% for 40 min. The fermented dough was then removed and divided into 300 g balls, each shaped into rounds and fermented again for a further 10 min. Thereafter, the dough was flattened and
shaped into circles with a thin center and thick circumference. A pattern was poked or stamped onto the raw Nang dough and, finally, sesame seeds were sprinkled onto the surface.

2.3.2. Freeze-thaw treatment

The Nang dough was frozen and thawed in accordance with the following freezing and thawing conditions based on industrialized flour products: freezing temperatures of -20°C, -30°C, -40°C, -50°C, and -60°C, respectively; freezing times of 30 min, 40 min, 50 min, 60 min and 70 min, respectively; thawing temperatures of 25°C, 30°C, 35°C, 40°C and 45°C, respectively; and thawing times of 10 min, 15 min, 20 min, 25 min and 30 min, respectively. The Nang dough was frozen in a low temperature refrigerator with set temperature, and after freezing, it was placed to be thawed in a ferment box with appropriate conditions.

2.3.3. Baking of Nang

After freezing and thawing, the raw Nang dough was baked in an oven at 180°C (top and bottom temperatures) for 21 min. After cooling, the sensory and textural properties of the baked Nang were measured.

2.3.4. Sensory quality assessment

After baking, the Nang was left to cool at room temperature for 1 hour, according to the methods described by Li et al. [24] and Zou et al. [25], and then sliced into small pieces approximately 20 mm high, to identify differences that may occur between the core (the thin center) and the thick edge of the product’s special structure. A total of 10 assessors evaluated the sensory quality of the baked Nang in terms of its color, flavor, organizational structure, hardness, crispness and chewiness.

| term               | evaluation standard                                           | score |
|--------------------|--------------------------------------------------------------|-------|
| color              | pale yellow or dark brown colour, not uniform                | 1-10  |
|                    | attractive baking color, lustrous, uniform color             | 11-20 |
| flavor             | fragrance of baking products and raw materials              | 1-10  |
|                    | rich smell, with raw and auxiliary materials unique flavor   | 11-20 |
| organizational      | Uneven, collapse, irregular profile                         | 1-7   |
| structure          | the structure is uniform from the core and edge sections    | 8-15  |
|                    | too soft or too hard                                        | 1-7   |
|                    | neither too hard, nor too soft                              | 8-15  |
|                    | not crisp or little                                         | 1-7   |
|                    | crisp                                                       | 8-15  |
|                    | short period of time required to chew once per second to    | 1-7   |
|                    | achieving swallowing granularity                            |       |
| chewiness           | long time required                                          | 8-15  |

2.3.5. Orthogonal test design and verification of the effects of freeze-thaw conditions on Nang quality

Single factor experiments were carried out for freeze-thaw temperature and time under different conditions, and the optimal processing parameters were selected through sensory evaluation, after which the orthogonal experiment was conducted. On the basis of the single factor screening conditions, orthogonal analysis with 4 factors and 3 levels of Lo (i) was used to conduct the optimization test, and the optimal processing conditions were determined based on the sensory score (Table 2). The optimal combination of the single factor experiment and the optimal combination of the orthogonal experiment were compared so as to select the group with the highest sensory assessment score for the final processing conditions.
| Level | A freezing temperatures/℃ | B freezing times/min | C thawing temperatures/℃ | D thawing times/min |
|-------|---------------------------|---------------------|-------------------------|---------------------|
| 1     | -30                       | 40                  | 30                      | 15                  |
| 2     | -40                       | 50                  | 35                      | 20                  |
| 3     | -50                       | 60                  | 40                      | 25                  |

2.3.6. Determination of texture properties of Nang
According to the method described by Li et al. [24], a texture analyzer was used to measure the hardness, springiness, cohesiveness and chewiness of the Nang core and Nang edge after baking and cooling at room temperature. Texture profile analysis (TPA) parameters were as follows: a TMS6 mm probe was used, the pre-test rate was 6.0 mm/s, the test rate was 6.0 mm/s, and the post-test rate was 6.0 mm/s; the trigger force was 0.1 N; the compression ratio was 40%, and the time interval between two compressions was 5 s. Analysis of each sample was repeated in triplicate.

2.3.7. Determination of flavor substances
Gas chromatography-ion mobility spectrometry (GC-IMS) was used to determine flavor substances in the baked and cooled Nang, according to the method described by Meng Xintao [26] with slight modifications. The conditions were as follows: headspace incubation temperature was 120℃; incubation time was 10 min; incubation speed was 500 r/min; headspace injection needle temperature was 105℃; injection volume was 1 mL in unshunted mode; cleaning time was 30 sec; the carrier gas was high purity nitrogen (≥99.99%); column temperature was 40℃; running time was 21 min at the flow rate of 2.00 mL/min for 1 min, increased linearly to 20.00 mL/min within 10 min, was and then increased linearly to 100.00 mL/min within 5 min followed by maintaining for 5 min. The samples in the headspace flask were incubated, the headspace components were extracted with a heated injector, and the volatile components were analyzed using the FlavourSpec GC-IMS.

2.4. Data processing
The qualitative analyses of the characteristic flavor compounds, difference chromatogram and fingerprint chromatogram were performed by means of Laboratory Analytical Viewer (LAV) GC-IMS equipment and GC-IMS Library Search analysis software, and SPSS Statistics V21.0 was used for statistical analysis. Experimental data were expressed as mean ± standard deviation (mean±SD). One-way analysis of variance (ANOVA) was used for the comparison between different groups, and Duncan’s test was used for post-comparison. In addition, $P<0.05$ was considered statistically significant.

3. Results and analysis

3.1. Influence of freeze-thaw conditions on the sensory quality of Nang

3.1.1. Influence of different freeze-thaw conditions
The sensory quality evaluation results of Nang under different freeze-thaw conditions were shown in Fig. 1. When the other three factors were fixed and one of the factors was changed, the sensory quality of Nang was affected significantly ($P<0.05$). When the freezing temperature was -30 to -50℃, the sensory quality score of the Nang was significantly higher than that in freezing temperatures of between -20℃ and -60℃. When the freezing time was between 40 min and 60 min, the sensory quality score of the Nang was significantly higher than that in freezing times of 30 min to 70 min. The effect of thawing temperature on the sensory quality of the Nang was also significant. The sensory quality score of Nang thawed at 35℃ was 85.20, which was significantly higher than that when thawed at 45℃, while the sensory quality score of Nang thawed for 25 min was 83.64, which was
significantly higher than that of the Nang thawed for 10 min (P<0.05). When the thawing temperature was too high and thawing time was too long, the flavor of the Nang dough was soured, but when the thawing temperature was too low and the thawing time was too short, a serious loss of moisture in the Nang was induced by the insufficient thawing before baking. Similar effects of thaw conditions on frozen dough have been reported by Yang et al. [8] who found that thawing by microwave could result in the serious loss of moisture in dough, and that dough thawed in cold or at room temperature also resulted in a larger volume of bread and a significant linear correlation (R²=0.9557) with viable yeast cells.

The fixed conditions other than single variables in the figure were freezing temperature -40 °C, freezing time 50 min, thawing temperature 35 °C and thawing time 20 min. Different letters represent a significant difference in each univariate among the groups (P<0.05).

3.1.2. Optimization of freeze-thaw treatment conditions

The sensory quality assessment scores under different freeze-thaw temperatures and times were presented in the radar chart (Fig. 2 and Table 3), in which it is evident that the color and flavor scores of experimental group 3 were the highest, while the flavor values of experimental groups 2, 7 and 8 were second only to those of experimental group 3. The tissue structure scores of experimental group 7 were the highest, and the hardness, crispness and chewiness scores of experimental group 8 were highest (Fig. 2). In contrast to the single-factor experimental results, the orthogonal optimization results showed that freezing temperature had the most significant effect on the sensory quality of the Nang, followed by thawing time and then freezing time, while thawing temperature having the least effect. The optimal combination of process conditions was found to -50°C freezing for 50 min and 40°C thawing for 20 min (Table 3). Moreover, the ANOVA results showed that freezing temperature had the most significant difference and the greatest impact on the sensory quality of Nang (Table 4). The sensory score of Nang under the optimized conditions was 81.26, indicating that the influence of freeze-thaw conditions on the sensory quality of Nang was the result of the combined action of all four factors. The lower freezing temperature, with a freezing time that was neither too short nor too long, were found to be the most favorable conditions to preserve the sensory quality of Nang, thus concurring with the mean trend of the effect of freezing treatment conditions on the sensory score of Nang in the single factor experiment. This phenomenon may be linked to the formation of ice crystals from water and the inactivation of the yeast. When the freezing temperature is lower than -40°C, ice crystals form rapidly and, consequently, are less harm to the dough’s network structure[27-28].
Fig. 2 Single score of sensory quality evaluation

Table 3 Orthogonal optimization results

| number | A freezing temperatures/℃ | B freezing times/min | C thawing temperatures/℃ | D thawing times/min | Score of sensory quality |
|--------|-----------------|----------------------|--------------------------|---------------------|--------------------------|
| 1      | -30             | 40                   | 30                       | 15                  | 76.50                    |
| 2      | -30             | 50                   | 35                       | 20                  | 79.70                    |
| 3      | -30             | 60                   | 40                       | 25                  | 79.20                    |
| 4      | -40             | 40                   | 35                       | 25                  | 76.20                    |
| 5      | -40             | 50                   | 40                       | 15                  | 77.11                    |
| 6      | -40             | 60                   | 30                       | 20                  | 77.67                    |
| 7      | -50             | 40                   | 40                       | 20                  | 82.00                    |
| 8      | -50             | 50                   | 30                       | 25                  | 82.56                    |
| 9      | -50             | 60                   | 35                       | 15                  | 80.00                    |
| K₁     | 78.47           | 78.23                | 78.91                    |                     | 77.87                    |
| K₂     | 76.99           | 79.79                | 78.63                    |                     | 79.79                    |
| K₃     | 81.52           | 78.96                | 79.44                    |                     | 79.32                    |
| R      | 4.53            | 1.56                 | 0.80                     |                     | 1.92                     |

Factor ranking optimal level: A>D>B>C

Optimal level: A₃B₂C₃D₂
Table 4 Analysis of variances

| source of variation          | Q     | f  | Variance | F     | Fα significant level |
|------------------------------|-------|----|----------|-------|----------------------|
| freezing temperatures        | 31.398| 2  | 15.699   | 13.384| F0.01 (2, 3) = 30.82 * |
| freezing times               | 3.641 | 2  | 1.8205   | 1.525 | F0.05 (2, 3) = 9.55   |
| thawing temperatures         | 0.999 | 2  | 0.4995   | 0.419 | F0.1 (2, 3) = 5.46    |
| thawing times                | 6.010 | 2  | 3.005    | 2.515 | F0.25 (2, 3) = 2.28   |
| composite error              | 42.63 |    |          |       |                      |

Notes: * indicates significant differences (P < 0.05).

3.2. Influence of freeze-thaw treatment on texture characteristics of Nang

TPA of Nang showed that the hardness, springiness, adhesiveness and chewiness of the Nang core and Nang edge were different under different freeze-thaw conditions (Table 5). The sensory scores of experimental groups 7, 8 and 9 were all above 80. Combined with the TPA results, it was found that the hardness scores of the Nang core and Nang edge were 15.5-20 g and 10-14 g, respectively; their springiness was 4.5-8% and 8-9%, respectively; adhesiveness was 13-15 and 10-11, respectively; chewiness was 66-98 g and 78-95 g respectively; and, lastly, the flavors under the freeze-thaw conditions in this range were good. These results suggest that the proper freezing rate can not only reduce the destructive effects of ice crystal formation to the internal structure of dough but also is beneficial to the maintenance of yeast activity. These findings are also consistent with those of previous reports. According to Le-Bail et al.[29] and Kondakci et al.[30], a freezing rate that was either too high or too low had adverse effects on the texture properties of fermented dough. Moreover, this research also ascertained that the hardness and adhesiveness of the Nang core were higher than those qualities at the Nang edge, while springiness and chewiness were higher at the edge than at its core.

Table 5 Effects of freeze-thaw treatment on TPA of Nang

| Nang core | Nang edge | Nang core | Nang edge | Nang core | Nang edge | Nang core | Nang edge | Nang core | Nang edge |
|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| hardness/g | springiness/% | cohesiveness | chewiness/g |
| 1  | 35.83±4.87  a | 15.13±3.11 ab | 4.45±0.24  e | 9.66±1.12 cd | 11.07±0.48 a | 4.17±0.24 e | 12.80±3.63 ab | 74.96±24.09  c | 119.40±20.45 a |
| 2  | 30.50±2.20  a | 7.05±0.88  a | 4.45±0.24  e | 9.66±1.12 cd | 11.07±0.48 a | 4.17±0.24 e | 12.80±3.63 ab | 74.96±24.09  c | 119.40±20.45 a |
| 3  | 20.93±0.69  d | 7.69±1.64 b | 4.48±0.46  e | 9.66±1.24 cd | 11.07±0.48 a | 4.17±0.24 e | 12.80±3.63 ab | 74.96±24.09  c | 119.40±20.45 a |
| 4  | 15.13±3.11 ab | 7.69±1.64 b | 4.48±0.46  e | 9.66±1.24 cd | 11.07±0.48 a | 4.17±0.24 e | 12.80±3.63 ab | 74.96±24.09  c | 119.40±20.45 a |
| 5  | 20.93±0.69  d | 7.69±1.64 b | 4.48±0.46  e | 9.66±1.24 cd | 11.07±0.48 a | 4.17±0.24 e | 12.80±3.63 ab | 74.96±24.09  c | 119.40±20.45 a |
| 6  | 20.93±0.69  d | 7.69±1.64 b | 4.48±0.46  e | 9.66±1.24 cd | 11.07±0.48 a | 4.17±0.24 e | 12.80±3.63 ab | 74.96±24.09  c | 119.40±20.45 a |
| 7  | 15.56±2.89  d | 10.31±2.15 ab | 7.49±1.37  d | 8.85±0.08  bc | 12.69±1.07  ab | 9.94±2.71  d | 78.21±8.93 b | 78.21±8.93 b |
| 8  | 20.20±0.78  a | 12.28±0.14 ab | 7.81±2.27  b | 8.17±0.65  cd | 14.96±1.22  bc | 9.50±0.35  d | 79.50±15.24 b | 79.50±15.24 b |
| 9  | 17.16±2.94  d | 13.73±3.28  ab | 4.53±0.37  d | 8.50±0.35  cd | 14.43±1.69  bc | 11.32±3.31  ab | 65.69±10.83 a | 95.20±24.58 d |

Note: different letters represent a significant difference in each univariate among the groups (P < 0.05).
3.3. Sensory quality evaluation and TPA correlation analysis

Further analysis of the correlations between the sensory qualities and TPA of Nang showed that the sensory score was negatively correlated with the hardness, springiness, cohesiveness and chewiness of both the Nang edge and Nang core, but was positively correlated with the springiness index (Table 6). There was a quite significant negative correlation between the flavor value of Nang and its TPA hardness value \( (P<0.001) \) and a significant negative correlation between the flavor value of Nang and its TPA cohesiveness \( (P<0.05) \); moreover, there was a significant positive correlation between the chewiness of the Nang edge and TPA springiness \( (P<0.05) \), which indicated that the sensory quality changes to the Nang edge caused by the freeze-thaw treatment were more obviously reflected in the differences in flavor and chewable taste. The sensory quality evaluation results of the Nang core and Nang edge were consistent (Table 7). From the significant positive correlation between color and crispness, and the total score in the sensory qualities of both core and edge, it is evident that color and crispness contribute significantly to the sensory quality of Nang. Secondly, in the sensory quality evaluation of the Nang core, there was a significant negative correlation between crispness and TPA hardness \( (P<0.001) \), and a significant negative correlation between crispness and cohesiveness \( (P<0.05) \), indicating that the freeze-thaw treatment led to significant changes in the hardness and adhesiveness of Nang, as reflected mostly in the sensory quality changes in its crispness. Thus, the Nang core was crisp but not hard, while the Nang edge had both good springiness and chewiness, resulting in higher sensory evaluation scores and, thereby, meeting the taste expectations of most consumers.

| Table 6 Correlation analysis of sensory quality evaluation with TPA of Nang edge |
|----------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                      | sensory qualities | TPA              |
|                                      | color            | flavor          | organizational structure | hardness | crispness | chewiness | score | hardness | springiness | cohesive ness | chewiness |
| color                                 | 1                |                 | 1                          |           |           |           |       |           |             |              |           |
| flavor                                | 0.755*           | 1               |                             |           |           |           |       |           |             |              |           |
| organizational structure              | 0.113            | 0.085           | 1                          |           |           |           |       |           |             |              |           |
| hardness                              | 0.314            | 0.217           | 0.223                      | 1          |           |           |       |           |             |              |           |
| crispness                             | 0.595            | 0.147           | 0.384                      | 0.403      | 1          |           |       |           |             |              |           |
| chewiness                             | 0.251            | 0.135           | 0.195                      | 0.482      | 0.442      | 1          |       |           |             |              |           |
| score                                 | 0.772*           | 0.569           | 0.489                      | 0.655      | 0.790*     | 0.652      | 1      |           |             |              |           |
| hardness                              | -0.525           | -0.841**        | -0.019                     | -0.162     | 0.188      | -0.107    | -0.334 | 1          |             |              |           |
| crispness                             | -0.217           | -0.076          | -0.268                     | -0.071     | -0.044     | 0.696*    | 0.030  | -0.040    | 1            |              |           |
| cohesiveness                          | 0.563            | -0.687*         | -0.006                     | -0.291     | 0.15       | 0.157     | -0.273 | 0.862**   | 0.371        |              |           |
| chewiness                             | -0.498           | -0.479          | -0.107                     | -0.222     | 0.037      | 0.482     | -0.163 | 0.565     | 0.745*       | 0.877**      | 1           |

Note:**the correlation was significant at the 0.01 level\( (P<0.001) \),*the correlation was significant at the 0.05 level\( (P<0.05) \)

| Table 7 Correlation analysis of sensory quality evaluation with TPA of Nang centre |
|----------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                      | sensory qualities | TPA              |
|                                      | color            | flavor          | organizational structure | hardness | crispness | chewiness | score | hardness | springiness | cohesive ness | chewiness |
| color                                 | 1                |                 | 1                          |           |           |           |       |           |             |              |           |
| flavor                                | 0.755*           | 1               |                             |           |           |           |       |           |             |              |           |
organizational structure 0.113 0.085 1
hardness 0.314 0.217 0.223 1
crispness 0.595 0.147 0.384 0.403 1
crunchiness 0.251 0.135 0.195 0.482 0.442 1
score 0.772* 0.569 0.489 0.655 0.790* 0.652 1
hardness -0.425 0.025 -0.231 -0.052 -0.816** 0.05 -0.406 1
springiness 0.373 0.114 0.332 0.55 0.492 -0.235 0.404 -0.517 1
cohesiveness -0.205 -0.078 -0.123 0.083 -0.676* -0.125 -0.328 0.655 -0.077 1
chewiness 0.002 -0.03 -0.005 0.257 -0.368 -0.418 -0.183 0.212 0.494 0.802** 1

Note:**the correlation was significant at the 0.01 level( P<0.001),*the correlation was significant at the 0.05 level( P<0.05)

3.4. Influence of freeze-thaw treatment on flavor substances of Nang

3.4.1. Comparison and analysis of ion migration chromatography and GC-IMS spectra of Nang flavor

The flavor substance changes in the Nang under different freezing temperatures (-30℃, -40℃ and -50℃), at the freezing time of 50 min, thawing temperature of 40℃ and thawing time of 20 min, were shown in Fig. 3. In the observation and comparison of the three-dimensional spectrum (Fig. 3A) and the two-dimensional top view (Fig. 3B) detected by GC-IMS, the retention time and drift time were compared from the red vertical line that represented the reaction ion peak (RIP) and the points on both sides that represented volatile organic compounds (blue points for low content and red points for high content). The retention index of each compound was calculated by using n-ketone C4~C10 as the reference of the external standard, and the volatile compounds were qualitatively analyzed. In the comparative match with the GC-IMS library, it was found that the volatile components of Nang could be separated well under different freezing temperatures and have obviously different characteristic spectral data.

[Diagram showing ion migration chromatography and GC-IMS spectra of Nang flavor under different freezing temperatures]

unfrozen -30℃ freezing -40℃ freezing -50℃ freezing
3.4.2. GC-IMS fingerprint analysis of Nang flavor

The characteristic flavor fingerprint of Nang was constructed for the qualitative analysis of 41 characteristic flavor compounds, and to ascertain the effect of freezing temperature on Nang flavor compounds (Fig. 4). Compared with the flavor substances in pizza dough, Nang lacks esters and carboxylic acids. Among the 41 flavor compounds identified and characterized, it was found that 26 compounds were not significantly affected by freezing temperature, but the remaining 15 compounds were either significantly increased or decreased. It is evident that the flavor substance components of the Nang dough after freezing treatment were similar to that without freezing treatment, but there were some obvious differences (Fig. 4). Among them, region A showed some flavor compounds were not found in the unfrozen Nang, including 2-heptanone, n-hexane, 2-pentanone and cyclohexanone, but were detected in frozen Nang. Region B showed some characteristic flavor substances, including styrene and 2-hexanone, which were identified in the unfrozen Nang, but either weakened or disappeared after freezing. Region C showed a higher concentration of characteristic substances, including 2-heptanone and n-hexane, which may be the main reason for the high flavor score at -30°C based on the sensory evaluation results. This suggested that the presence of these flavor substances provided the tasters with a more pleasant taste experience. At -40°C, in comparison to the other two freezing temperatures, there was no production of obvious characteristic flavor substances. The characteristic substances in Region D were produced under the freezing condition of -50°C, including 2, 3-pentanedione, 2, 5-dimethylpyrazine, 2-ethyl-1-hexanol, 4-hydroxy-2, 5-dimethyl-3 (2H) -furanone, heptanol and 1-butanol. This may be because low-temperature freezing is helpful in maintaining the flavor substances formed in Nang dough during fermentation. The score of the Nang flavor dominated by the freezing temperature of -50°C is, thus, second only to that of -30°C in the sensory evaluation.
3.4.3. PCA analysis of Nang flavor substances

The qualitative characteristic flavor of Nang was the basis upon which the PCA analysis was carried out to compare the differences between different freezing temperatures (Fig. 5). The sum of the contribution rates of principal components 1 and 2 reached 73%. The characteristic flavor substances of the unfrozen and frozen Nang were clearly separated at -50℃, while those at the freezing temperatures of -30℃ and -40℃ were not as obviously separated and had higher aggregation degrees among the samples, which was consistent with the results of the fingerprint. Meanwhile, the differences of the freezing temperature of -50℃ from -30℃ and -40℃ further speculated the fingerprint results mentioned above that the lower freezing temperature was more conducive to maintain the flavor substances formed during the fermentation of Nang dough. The comparative results of the PCA analysis of the unfrozen and frozen Nang indicated that the freezing treatment did affect the special flavor of Nang under the precondition of maintaining sensory quality.

4. Conclusions

In this study, different freeze-thaw temperatures and times were found to affect the sensory quality of Nang, with the freezing temperature exerting a greater effect than the freezing time, followed by the thawing time and temperature. The orthogonal experiment showed the optimal freeze-thaw treatment conditions to be freezing at -50℃ for 50 min and thawing at 40℃ for 20 min. The TPA characteristics
revealed the hardness values of the Nang core and the Nang edge with freeze-thaw treatment were in the range of 15.5-20 g and 10-14 g, respectively, while the springiness values were 4.5-8% and 8-9%, respectively; the cohesiveness values were 13-15 and 10-11, respectively; the chewiness was between 66-98 g and 78-95 g, respectively. Correlation analysis showed that the effects of freeze-thaw treatment on the sensory quality of the Nang edge was the obvious changes in flavor and chewing texture, while the hardness and viscosity of Nang were most relevant to crispiness. The subsequent investigation of freezing temperatures had the most significant effect on flavor substances. Although similar compositions of flavor substances were observed between frozen and unfrozen Nang, more flavor compounds were found at the lowest tested freezing temperature of -50°C, including 2,3-pentanedione, 2,5-dimethylpyrazine, 2-ethyl-1-hexanol, 4-hydroxy-2, 5-dimethyl-3 (2H) -furanone, heptanol and 1-butanol.

Acknowledgments

This article is supported by Key research and development project (nky2019033) from the government of Xinjiang Uyghur Autonomous Region. Key research and development project of Xinjiang Autonomous Region.

References

[1] Wang, X. X., Yu, X. L., Hu, Z. Y., Wu, B. C., Zhao, L. (2015) Research status and improvement advance on the frozen flour product. Food and Grease, 28(07):5-8.
[2] Zhong, C. Research status and improvement progress of frozen flour products. (2020) Food Safety Bulletin, (21):188.
[3] Li, L. L., Guo, S. T. Development and problems of quick-frozen food industry in china. (2010) Science and Technology of Food Industry, 113(7):422-424.
[4] Barcenas, M. E., Bendito, C., Rosell, C. M. Use of hydrocolloids as bread improvers in interrupted baking process with frozen storage. (2004) Food Hydrocolloids, 18(5): 769-774.
[5] Meziani, S., Jasnjewski, J., Ribotta, P., Arab-Tehrany, E., Muller, J.M. Influence of yeast and frozen storage on rheological, structural and microbial quality of frozen sweet dough. (2012) Journal of Food Engineering, 109(3):538-544.
[6] Tao, H., Wang, P., Wu, F. F., Jin, Z. Y., Xu, X. M. Effect of freezing rate on rheological, thermal and structural properties of frozen wheat starch. (2016) RSC Advances, 6(100): 97907-97911.
[7] Lu, L., Xing, J. J., Yang, Z., Guo, X. N., Zhu, K. X. Enhancing the freezing-thawing tolerance of frozen dough using e-poly-L-lysine treated yeast. (2020) Food Bioscience, 37:1-7.
[8] Yang, S. K., Jeong, S., Lee, S. Elucidation of rheological properties and baking performance of frozen doughs under different thawing conditions. (2020) Journal of Food Engineering, 284.
[9] Yi, J., Kerr, W. L. Combined effects of dough freezing and storage conditions on bread quality factors. (2009) Journal of Food Engineering, 93, 495-501.
[10] Yi, J., Kerr, W. L. Combined effects of freezing rate, storage temperature and time on bread dough and baking properties. (2009) Food Science and Technology, 42(9):1474-1483.
[11] Akbarian, M., Koocheki, A., Mohebbi, M., Milani, E. Rheo-logical properties and bread quality of frozen sweet dough with added xanthan and different freezing rate. (2016) Journal of Food Science and Technology, 53(10): 3761-3769.
[12] Olivera, D. F., Salvadori, V. O. Effect of freezing rate intestinal and rheological characteristics of frozen cooked organic pasta. (2009) Journal of Food Engineering, 90(2):271-276.
[13] Leray, G., Oliete, B., Mezaize, S., Chevallier, S., Lamballerie, M. D. Effects of freezing and frozen storage conditions on the rheological properties of different formulations of non-yeasted wheat and gluten-free bread dough. (2010) Journal of Food Engineering, 100(1):70-76.
[14] Zhang, Y. Y., Li, Y. L., Liu, Y., Zhang, H. Effects of multiple freeze-thaw cycles on the quality of frozen dough. (2018) Cereal Chemistry, 95(4):499-507.
[15] Du, H. R., Zheng, X. L., Han, X. X., Zhang, J., Li, L. M., Liu, C., Bian, K. Effects of freezing conditions and thawing methods on the qualities of emptins frozen dough for steamed bread. (2015) Food and Feed Industry, 38(5):14-18.

[16] Liu, Y., Wang, X. Q., Fan, L. L., Zhang, X. L., Li, R., Gao, S. Effects of frozen storage time on quality and cooking characteristics of frozen dumpling. (2019) Agricultural Product Processing, (05):9-11.

[17] Wang, N., Pan Z. L., Kang, Y. C., Suo, B., Xie, X. H., Ai, Z. L. Study on effect of freezing energy-saving technology on quality of quick-frozen cooked noodle. (2020) Journal of Jiangxi Agricultural, 32(05):88-92+98.

[18] Zhang, J., An, Y. X., Wang, Y. Y., Hu, G. P. Quality change of frozen corn noodles during frozen storage. (2018) Food and feed industry, (12):24-27, 31.

[19] Li, X. X. The development of frozen steamed pizza dough. (2015) Food Technology, 40(6):186-189.

[20] Xiao, R. Y., Li, M., Hou, P. Present Situation and application prospect of frozen dough. (2019) Technology Information, 17(32):63+65.

[21] Abduuaizezi, A., Mamatreixiati, M., Aili, R., Kaiserj, A. Processing technique of nang and principles of nang baking machine. (2015) Anhui Agricultural Science, 43(18):286-288.

[22] Sun, Q. “Nang” and uygur society from perspective of silk-road culture. (2018) Journal of Central South University for Nationalities (Humanities and Social Sciences Edition), 38(03):84-88.

[23] Liu, J. L., Bai, Y. J., Feng, Z. S., Ayiguli, A., Shu, Y. J., Yang, M. J. Study on the optimum recipe of sprouted wheat flour nang. (2020) Chinese Food Additives, 31(11):28-38.

[24] Li, F., Jiang, X. F., Xie, Q., Ren, W. W., Zhao, G. M., Kong, L. M. Study of correlation between TPA parameters and sensory evaluation index of nang products. (2016) Food Industry, 37(03):205-208.

[25] Zou, S. P., Zhang, Q., Meng, Y. N., Ma, Y., Xu, M. Q., Tai, X. L., Tong, L. T. Effect of hot blanching on the quality of nang(baked crusty pancake) added with high proportion of raw dehydrated potato flour. (2020) Food Science, 41(16):52-56.

[26] Meng, X. T., Qiao, X., Pan, Y., Zou, S. P., Zhang, T., Zhang, Q. Characteristic flavor compounds fingerprinting of mutton from different producing regions of Xinjiang, China by Gas chromatography-Ion mobility spectrometry. (2020) Food Science, 41(16):218-226.

[27] Xin, C., Nie, L. J., Chen, H. L. Effect of degree of substitution of carboxymethyl cellulose sodium on the state of water, rheological and baking performance of frozen bread dough. (2018) Food Hydrocolloids, 80,8-14.

[28] Park, J. I., Grant, C. M., Atifield, P. V., Dawes, I. W. The freeze-thaw stress response of the yeast saccharomyces cerevisiae is growth phase specific and is controlled by nutritional state via the RAS-cyclic AMP signal transduction pathway. (1997) Appl Environ Microbiol, 63(10), 3818-3824.

[29] Le-bail, A., Nicolitch, C., Vuillod, C. Fermented frozen dough: impact of pre-fermentation time and of freezing rate for a pre-fermented frozen dough on final volume of the bread. (2010) Food and Bioprocess Technology, 3(2):197-203.

[30] Kondakci, T., Zhang, J. W., Zhou, W. B. Impact of flour protein content and freezing conditions on the quality of frozen dough and corresponding steamed bread. (2015) Food and Bioprocess Technology, 8(9):1877-1889.

[31] Jiang, Y. H., Jia, H. F., Deng, H. Analysis of volatile flavor compounds in pizza produced by normal and frozen dough. (2017) Food and Grease, 30(09):71-73.