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Study on the effects of different ammonium salts on baked bread

Ruiqi Sun, Jiaqi Zheng, Mengdi Niu, Jun Wang

College of Food Science & Nutritional Engineering, China Agricultural University, Beijing, China

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ABSTRACT

Three different ammonium salts, namely diammonium hydrogen phosphate, ammonium bicarbonate, and ammonium carbonate, were added into bread samples as an additive to analyze their effects on bread. The color, texture, deoxyfructosazine of the functional substance, and pyrazine flavor substance, which were closely related to the quality of the bread, were analyzed. The addition of ammonium salts during the preparation of bread led to the darkening and hardening of the bread. Meanwhile, compared with the control group, the Maillard reaction between the ammonium salt and reducing sugar in bread produced functional deoxyfructosazine and pyrazine flavor substances. Among the three ammonium salts, the addition of diammonium hydrogen phosphate at different concentrations had the most substantial effect on the quality of baked bread, including the production of more deoxyfructosazine, and more types of pyrazine flavor substances. Through an analysis of the value of odor activity, it was found that the addition of diammonium hydrogen phosphate had a more remarkable contribution to the flavor of the bread. The maximum total content of deoxyfructosazine reached 1292.23 μ g/g, and the value of odor activity reached 39.86 in this study. These results are extremely useful in the production of bread with superior flavor and functional characteristics. Also, they provide a guideline for the selection of ammonium salt as an additive in baked goods.

1. Introduction

As an important staple food, bread is a kind of baked food which is mainly processed with grain-ground powder. Studies have shown that the average person consumes 100–200 g of bread on a daily basis [1]. Therefore, bread is also one of the key nutrient sources [2].

In the process of bread-making, reducing sugar is often used, and high-fructose corn syrup has gradually replaced sucrose in the production of bread. This is because of its good fermentability, coking, and moisturizing properties with appropriate sweetness [3–5]. In addition, ammonium salts can be used as a kind of additive in the baking of bread, which is compliant with *the Chinese National Standard for Food Safety Standard on the Use of Food Additives* (GB 2760-2014) and *the Codex Alimentarius Commission* (CAC) standards. For instance, diammonium hydrogen phosphate can be used for moisture retention and as an acidity regulator and leavening agent in bread processing. Ammonium bicarbonate and ammonium carbonate can also be used as leavening agents in the production of certain types of bread. At the same time, these ammonium salts act as a nitrogen source to feed yeast, which facilitates fermentation [6,7].

In this case, in a previous study, ammonium salts and reducing sugar are used in combination, and when the bread is baked, the

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^{*} Corresponding author. College of Food Science & Nutritional Engineering, China Agricultural University, Beijing, 100083, China.

E-mail addresses: ricky77q@163.com (R. Sun), 903067957@qq.com (J. Zheng), niumd928@163.com (M. Niu), wangjun1@cau.edu.cn (J. Wang).

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Maillard reaction occurred. The reaction, on one hand, improves the color and flavor of the bread [8,9]. On the other hand, in an earlier study, it was proved that ammonium can easily react with reducing sugars such as glucose and fructose to produce pyrazines [10], such as deoxyfructosazines (DOF), including 2,5-deoxyfructosazine (2,5-DOF) and 2,6-deoxyfructosazine (2,6-DOF). However, this study used reactants that were reacting in a solution and did not demonstrate the actual process of the reaction exhibited by the reactants in a food system.

There were a series of studies on the reaction of glucose with ammonia and its compounds were carried out to explain the formation mechanism of 2,5-DOF and 2,6-DOF in such reactions. The DOFs were stable, non-volatile, and did not have any flavors in them. However, the DOFs performed pyrolysis under a high temperature and yielded some small molecules of substances with a pyrazine flavor with obvious fragrance characteristics, which would have a certain influence on the flavor characteristics of the food. In certain studies, the presence of deoxyfructosazines has been identified in roasted peanuts, caramel, and soy sauce [11–13], It was reported that the DOFs exhibited certain functional effects and biological activities, including inhibiting the growth of T-cell interleukin-2, which can help the immune system fight inflammation[14]. Furthermore, DOF exhibited the function of regulating blood sugar and assistance in treating diabetes [15].

In several cases, both ammonium salts and reducing sugar were used in bread-making. At present, fragrant and functional bread is quite prevalent in food production and consumption [16,17]. Using ammonium salts and reducing sugar simultaneously in bread is an effective way to produce this kind of bread. However, there is a lack of research on the reaction of these substances under baking conditions, especially on the formation of functional DOFs and the resulting pyrazine-flavor compounds, as well as the effect on the other characteristics of bread. The rules of the reaction play an important role in the selection of the ammonium salt and the control of the flavor and function, including the color and texture of the bread.

2. Materials and methods

2.1. Materials and chemicals

High-gluten flour was purchased from Beijing Guchuan Food Co. Ltd (Beijing, China), which was produced in line with GB/T 8607. The butter was purchased from Shanghai Gough Food Co., Ltd (Shanghai, China, the butter from fresh milk); edible sodium chloride was bought from China National Salt Industry Corporation (Beijing, China); edible fructose was bought from Zibo Prebiotic edge Biotechnology Co., Ltd (Shandong, China); edible glucose was purchased from Weifang revitalizing coke Co., Ltd (Shandong, China); and yeast powder was obtained from Angel Yeast Co. Ltd (Beijing, China). Diammonium hydrogen phosphate (\geq 99 % purity), and ammonium carbonate (\geq 99 % purity) were of food grade and were obtained from Henan Kaiwen products Co., Ltd (Henan, China).

The 2-octanol was of HPLC grade and obtained from Shanghai Maclin Biochemical Technology Co., Ltd. 2,5-DOF (\geq 95 % purity) and 2,6-DOF (\geq 99 % purity) were purchased from J&K Scientific Co., Ltd. Acetonitrile was of HPLC grade and purchased from J&K Scientific Co., Ltd.

2.2. Preparation of bread samples

Baked bread samples were produced with 240.0 g high-gluten flour, 2.4 g edible sodium chloride, 24.0 g sugar (using 12.0 g fructose and 12.0 g glucose to simulate 1:1 high-fructose corn syrup), 2.4 g yeast powder (it was mixed evenly with 120 mL water at 40 °C), and a certain amount of ammonium salt. The bread baked without the ammonium salts was control sample. Three kinds of ammonium salts were used as additive, which were diammonium hydrogen phosphate, ammonium bicarbonate, and ammonium carbonate. The content of each was maintained at the high, medium, and low level. Among them, the "high" content was 80 % of the maximum limit of use of these ammonium salts that are used as additives in baking products in the Chinese National Standard for Food Safety Standard on the Use of Food Additives (GB 2760-2014). "Medium" was defined as 1/2 of the "high" content, and "low" was defined as 1/2 of the "medium" content. In the bread samples used in this study, the highest addition amount of diammonium phosphate was 4.94 g, ammonium bicarbonate was 5.78 g, and ammonium carbonate was 3.59 g. The concrete ammonium concentration of the three salts was the same, and the electronic analytical balance (0.0001, Sartorius, Germany) was used for weighing. The abovementioned ingredients were mixed into a dough with a dough mixer (ASM-DA600, Appliance Co. of America, USA), and subsequently, 10.0 g butter was added and mixed with a dough mixer to form a smooth surface. The dough was put into the oven (T4-L326F, Midea, China) and fermented at 30 °C for 1 h. Subsequently, the dough was taken out, kneaded, and vented, and divided into three pieces of the same weight (134 \pm 2 g), which were placed in the same toast mold (7.5 cm \times 7.5 cm \times 7.5 cm) and fermented at 30 °C for 30 min. Following this, the toast mold was covered, the doughs were baked in the oven at 160 °C for 30 min. The weight of each bread after baking was about 128 ± 2 g, and subsequently, the bread samples were removed from the mold and kept at room temperature for 2 h before being tested.

2.3. Analysis of bread samples prepared with different ammonium salts

2.3.1. Determination of color

The objective color (L^* - lightness, a^* - redness, and b^* - yellowness) of the bread samples was measured using a chroma meter (CR-400, Konica Minolta, Japan). The L*, a^* , and b^* values for each group were analyzed as the average of three readings. The total color difference ΔE between the control and the sample bread with ammonium salt was calculated according to the methods described by

Ref. [18], with
$$\Delta E = \sqrt{\left(L^* - L_0^*\right)^2 + \left(a^* - a_0^*\right)^2 + \left(b^* - b_0^*\right)^2}$$
.

2.3.2. Determination of texture

The bread samples were sliced into pieces with thicknesses of 20 mm. The texture analyzer (CT3, Brookfield, AMETEK, USA) was used for detecting the texture. The probe used was TA-AACC36, the trigger load was 5.0 g, the pre-test speed was 2 mm/s, the test speed was 1 mm/s, the post-test speed was 1 mm/s, and the compression rate was 40 %.

2.3.3. Determination of DOF content

The weight of the bread samples was determined to be 5.0 g. Subsequently, they were mixed with 40 mL water with ultrasonic treatment for 30 min. The solution was centrifuged at a speed of $3000 \text{ r}\cdot\text{min}^{-1}$ for 10 min to obtain the supernatant and transferred to a volumetric flask of 50 mL. Finally, the solution was set at a volume of 50 mL and filtered through a filter membrane of 0.22 µm to obtain the test samples. These test samples were analyzed by the HPLC system equipped with an ultraviolet detector (SPD20-A, Shimadzu, Japan). An amino column (Shim-pack GIST NH₂, 4.6 × 250 mm, 5 µm) was used to analyze the DOF contents, and the column temperature was set at 30 °C. The injection volume was set at 5 µL. The mobile phase was a 20/80 (v/v) isocratic mixture of water and acetonitrile, and the flow rate was 0.4 mL/min. Each cycle lasted 30 min, and the data were analyzed using the LabSolutions system Version 5.98 software.

The content of the two kinds of DOF in various bread samples was calculated according to the standard curves of 2,5-DOF and 2,6-DOF, and subsequently, they were summed to obtain the total DOF content.

2.3.4. Analysis of flavor compounds

The flavor compounds were extracted and detected through solid-phase microextraction (SPME) combined with the analysis of gas chromatography-mass spectrometry (GCMS-TQ8050 NX, Shimadzu, Japan). Each sample (2.0 g) was weighed into a headspace vial of 20 mL and equilibrated at 60 °C in a water bath for 10 min. The VAR/DVB/PDMS fiber (Supelco, PA, USA) of 50 μ m was inserted into the headspace of the vial over the samples. The flavor compounds were extracted for 40 min. Subsequently, the fiber was inserted into the injection port of GC at 250 °C for 3 min for desorption.

The flavor compounds were analyzed through GC–MS using a DB-WAX122-7032 Capillary Column (60 m \times 0.25 mm \times 0.25 μ m, Shimadzu) under the following conditions. The flow rate of helium used as the carrier gas was 1 mL/min; the oven temperature was

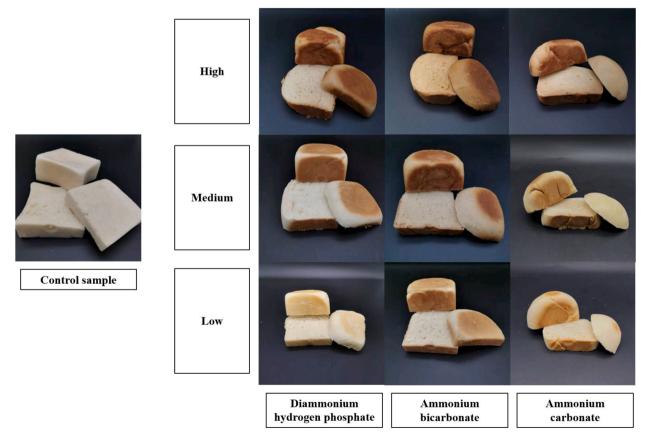


Fig. 1. Appearance of bread samples with different types and amounts of ammonium salts.

maintained at 40 °C for 2 min, increased to 230 °C at a rate of 5 °C/min, and subsequently, held for 10 min at the temperature; the ionization mode used was the electron impact ion source (EI); the ion source temperature was 200 °C; the electron energy was 70 eV; and the scan range was from 33 to 495 m/z for detecting all the compounds. The flavor compounds were identified by comparing the mass spectra with those included in the National Institute of Standards and Technology (NIST, MD, USA) library.

2-octanol was used as the internal standard to obtain the content of each pyrazine flavor substance produced by different groups, and subsequently, their content and threshold values were used to calculate their respective values of odor activity, and then added. The formula used is expressed as follows.

 $OAV = S_1 \times C_0 \times V_0 / C_{thr} \times S_0 \times M$

OAV: the value of odor activity;

 S_1 : the peak area of the flavor compounds;

 C_0 : the concentration of the internal standard, $\mu g/mL$;

*V*₀: the additive amount of the internal standard, mL;

 S_0 : the peak area of the internal standard;

M: the weight of the samples, g;

 C_{thr} : threshold of pyrazine flavor substances in water, mg/m³ [19].

3. Results and discussion

3.1. Chromatic aberration of bread samples

The color of bread, especially the crust and internal color, is an important reflection of the quality of bread, which directly affects consumers' preferences to bread. Therefore, the color is vital to evaluate the quality of bread during baking [20,21]. When different ammonium salts are used as leavening agents in bread, the Maillard reaction will occur between the ammonium salts and reducing sugar in bread, which will affect the color of the bread. Three ammonium salts at different concentrations were investigated, and the appearance of baked bread is shown in Fig. 1. It is clear that the addition of different types and concentrations of ammonium salts during bread preparation will produce a diverse appearance for the bread, especially in color [22]. The larger the amount of ammonium salts added, the darker the color of the skin of the bread sample. The color of the inside portion of the bread also had a similar change in depth, but it was not as obvious as the change in the color of the bread skin. For the bread samples with the same level of ammonium salt, the color of the inside and outside portion of the bread is also different because of the different types of ammonium salts added.

The outer surface and center portion of the bread samples were analyzed for chromatic aberration. The results are shown in Table 1. Values of ΔE >3 were observed, which indicate that this color difference is noticeable to the human eye [23]. For the crust of the sample bread, compared with the control bread sample without the addition of an ammonium salt, the value of ΔE >3 was observed on the addition of three kinds of ammonium salt at the high, middle, and low levels. This indicates that the addition of these several kinds of ammonium salts in the bread has a visible effect on the color of the crust of the sample bread.

As can be observed from Table 1, for the sample bread with three different ammonium salts added, the L* value of the crust of the

Table 1

Chromatic aberration of bread samples with different types and amounts of ammonium salts (n = 3).

Category		The crust of bread				The internal of bread			
		L*	a *	b*	ΔΕ	L*	a *	b*	ΔE
Control sample		$81.40 \ \pm$	1.20 ± 0.34	$22.90~\pm$		$\textbf{78.00} \pm$	$-2.13~\pm$	14.09 \pm	
		1.18		0.60		2.18	0.13	0.60	
Diammonium hydrogen	High	49.61 \pm	19.35 \pm	36.86 \pm	39.18	76.65 \pm	$-0.78~\pm$	$20.35~\pm$	6.54
phosphate		2.23	0.53	1.24		0.41	0.48	1.20	
	Medium	72.93 \pm	$\textbf{6.49} \pm \textbf{6.46}$	31.79 \pm	13.37	80.28 \pm	$-2.41~\pm$	15.48 \pm	2.68
		8.49		8.21		1.15	0.21	1.26	
	Low	82.00 \pm	$-2.23~\pm$	$\textbf{18.88} \pm$	5.32	83.71 \pm	$-2.45~\pm$	14.16 \pm	5.72
		1.10	0.12	1.09		0.45	0.19	0.83	
Ammonium bicarbonate	High	45.74 \pm	15.17 \pm	$24.92~\pm$	38.35	66.68 \pm	2.20 ± 0.59	$20.80~\pm$	13.85
		1.07	1.24	1.66		1.64		1.70	
	Medium	50.69 \pm	$\textbf{7.83} \pm \textbf{0.63}$	24.72 \pm	31.48	77.26 \pm	$-2.15~\pm$	19.28 \pm	5.24
		2.10		0.75		1.14	0.44	1.79	
	Low	79.53 \pm	$-1.14~\pm$	$20.34~\pm$	3.94	78.25 \pm	$-2.77~\pm$	16.82 \pm	2.82
		0.48	0.18	0.74		0.46	0.99	0.65	
Ammonium carbonate	High	74.65 \pm	2.91 ± 0.70	$24.93~\pm$	7.25	71.38 \pm	$-1.02~\pm$	19.55 \pm	8.65
		2.50		0.96		1.60	0.35	0.55	
	Medium	76.53 \pm	2.72 ± 0.33	$21.98~\pm$	5.16	74.61 \pm	$-0.69~\pm$	17.77 \pm	5.21
		3.84		0.59		0.33	0.07	1.71	
	Low	$81.97~\pm$	$-1.73~\pm$	$20.22~\pm$	4.01	80.07 \pm	$-2.40~\pm$	17.87 \pm	4.32
		0.55	0.40	0.44		0.57	0.23	0.43	

bread samples increased when the amount of ammonium salts decreased. This indicates that the color brightness of the crust of the bread sample increased, which was consistent with the observations in Fig. 1. When the amount of ammonium salts increased, the degree of the Maillard reaction with reducing sugar in the bread deepened, and more Maillard reactions occurred, making the bread sample darker [9]. However, for the inside portion of the bread, although there was a similar change, the degree of change was less. For instance, in case of bread samples supplemented with diammonium hydrogen phosphate, the samples with "high" and "low" contents of ammonium salt demonstrated L^* values of 49.61 and 82.00, respectively, while the inner L^* values were 76.65 and 83.71, respectively. It is clear that the change in the ammonium salt content has a greater effect on the L^* value of the bread crust than the L^* value of the bread interior. It is because the outside of the bread is more exposed to air than the inside portion, which facilitates the evaporation of water and enables the reactants to have a more suitable water activity for the Maillard reaction.

In addition, with the decrease in the amount of the ammonium salt, the a^* value and b^* value of the bread samples with added contents of diammonium hydrogen phosphate and ammonium bicarbonate decreased. This indicates that when the amount of these two ammonium salts added is higher, the crust and internal color of the bread samples are more red and yellow in color, which is also consistent with the obtained images of the bread samples. However, for the bread samples supplemented with ammonium carbonate, the correlation between the change in a^* and b^* values and the use of ammonium salts was not obvious.

3.2. Texture of bread samples

The texture characteristics play an important role in determining the quality of bread. The application of functional substances or the change in processing technology might cause changes in the texture of the bread [24–26]. Therefore, we determined the texture of the bread samples with added ammonium salts and compared it with the control sample. The statistical tests of texture characteristics were performed using SPSS (version 27, IBM, USA). Data were expressed as mean value \pm standard deviation (SD). The results are illustrated in Table 2. One-way analysis of variance (ANOVA) was used to compare the difference among bread samples.

According to the measurement results, the bread sample with ammonium carbonate was observed to be the hardest, followed by that with ammonium bicarbonate, and that with diammonium hydrogen phosphate being the least hard. Moreover, the higher the dosage, the greater the hardness of the bread samples when the same kind of ammonium salt was added. When the same amount of ammonium salt was added, there were significant differences in the hardness, elasticity and viscosity of the bread samples with three different ammonium salts added (P < 0.05). When the same ammonium salt was used, the hardness and viscosity of the bread samples with different ammonium salt contents were significantly different (P < 0.05), but there were no significant differences in the elasticity of bread samples with different ammonium salt contents of ammonium carbonate (P > 0.05).

Furthermore, compared with the bread samples of blank control without ammonium salts, the hardness and viscosity of the nine groups of samples became greater. In the process of bread-baking, the products of the Maillard reaction, including two carbonyl compounds, would be coupled with gluten, which might improve the strength of gluten, thus showing an increase in hardness and viscosity [27].

3.3. DOF content of bread samples

The DOF is an important functional substance that can be produced in the bread via the reaction of ammonium salts and reducing sugar [28,29]. Through HPLC analysis, the chromatogram was obtained as shown in Fig. 2. It can be observed that the control bread samples did not generate the DOF, while the several breads with three kinds of added ammonium salts generated 2,5-DOF and 2,6-DOF to varying degrees.

As shown in Fig. 3, the DOF content in the bread samples increased with the increase in the usage of ammonium salt. In addition, under the same concentration of ammonium ion, the content of the DOF in the bread samples obtained by adding different ammonium salts was as follows: the bread samples with diammonium hydrogen phosphate produced more DOF than the bread samples with ammonium bicarbonate or ammonium carbonate.

When all the three ammonium salts were added at "high" levels, the total DOF content of the bread samples with added diammonium hydrogen phosphate was 3.74 times that of the bread samples with ammonium bicarbonate and 6.46 times that of the bread samples with ammonium carbonate. At the "medium" level, the total DOF of the bread samples with added diammonium hydrogen

Table 2

Texture of bread samples with different types and amounts of ammonium salts (n = 3).

Category		Hardness/g	Elasticity/g	Viscosity/g
Diammonium hydrogen phosphate	High	1343.17 ± 220.26	0.33 ± 0.01	999.53 ± 163.78
	Middle	1126.67 ± 80.06	$\textbf{0.40}\pm\textbf{0.03}$	524.40 ± 63.50
	Low	648.33 ± 156.71	0.32 ± 0.01	860.37 ± 123.59
Ammonium bicarbonate	High	2764.33 ± 326.96	0.30 ± 0.02	1559.30 ± 232.29
	Middle	2136.00 ± 189.58	0.32 ± 0.02	2110.03 ± 122.57
	Low	1165.17 ± 61.12	0.37 ± 0.01	894.20 ± 47.20
Ammonium carbonate	High	23817.67 ± 148.16	0.57 ± 0.09	1806.56 ± 40.30
	Middle	18207.50 ± 219.30	0.47 ± 0.05	1811.09 ± 60.76
	Low	1769.6 ± 61.65	0.42 ± 0.03	1435.80 ± 54.28
Control sample		$\textbf{469.83} \pm \textbf{44.72}$	0.41 ± 0.02	$\textbf{387.97} \pm \textbf{22.27}$

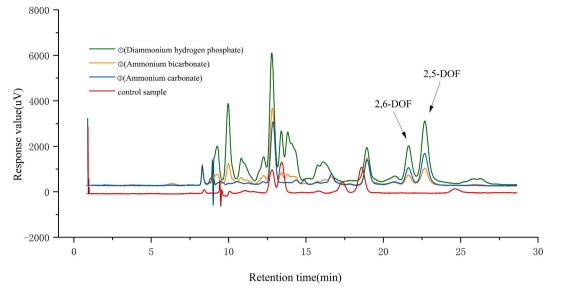
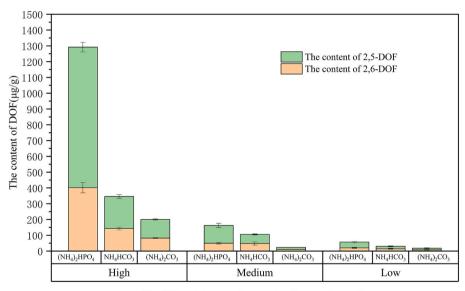


Fig. 2. HPLC chromatograms of bread samples with different types and amounts of ammonium salts.



The different types and amounts of ammonium salts

Fig. 3. The content of DOF of bread samples with different types and amounts of ammonium salts. Note: The error lines in the figure are for the content of 2,5-DOF and 2,6-DOF respectively.

phosphate was 1.53 times that of the bread sample with ammonium bicarbonate and 6.91 times that of the bread sample with ammonium carbonate. When the addition of the three ammonium salts was at the "low" level, the total DOF in the bread samples with diammonium hydrogen phosphate was still the largest, which was 1.79 times that of the bread samples with ammonium bicarbonate and 2.98 times that of the bread samples with added ammonium carbonate. The results indicate that diammonium hydrogen phosphate is more likely to react with fructose and glucose to produce DOF, which is due to the role of anions in the reaction between reducing sugars and ammonium salts. This may occur because the –OH and –O parts of the phosphate can cause phosphoric acid to act as an acid and base group at the same time, catalyzing the ring-opening of _D-glucopyranose [10].

In addition, we found that when the added amount of ammonium salt was at a "high" or "medium" level, a very significant difference (P < 0.01) was observed among the total DOFs of the bread samples with different kinds of added ammonium salts. However, when the added amount of ammonium salt was at a "low" level, the total DOFs of the bread samples with different kinds of added ammonium salts did not exhibit a highly significant difference (P > 0.01). The phenomenon is observed due to the excessive amount of reducing sugar in the process of bread-making. When the content of the ammonium salt was relatively low, the content of the reducing

sugar was far too much, which can cause the three ammonium salts to react with the reducing sugar as fully as possible. Therefore, for different types of ammonium salts, a little difference was observed in the total amount of the DOF produced when the contents of the ammonium salts were at a "low" level.

The fructose mainly reacts with ammonium salts to form 2, 5-DOF, while glucose mainly reacts with ammonium salts to form 2, 6-DOF [13,30,31]. The contents of 2,5-DOF and 2,6-DOF in bread samples with different ammonium salts were determined. The results show that for 9 groups of bread samples with varied types and amounts of added ammonium salts, the ratio of 2,5-DOF to 2,6-DOF generated by fructose and glucose with ammonium salts, respectively, was between 1.08 and 2.22 when the ratio of fructose and glucose was 1: 1. This indicates that the reactivity of fructose with ammonium salt was higher than that of glucose. In addition, it can be observed from Fig. 3 that the content ratio of 2, 5-DOF to 2, 6-DOF produced from diammonium hydrogen phosphate was the highest, which further implied that diammonium hydrogen phosphate has the highest reactivity among the three ammonium salts. The rule of DOF formation provides a reference for the selection of ammonium salt and reducing sugar for the production of bread.

3.4. Pyrazine substances in bread samples

Normally, different flavors are present in pyrazines, such as nutty, sweet, cocoa, coffee, and other flavors, providing rich flavor characteristics for food, especially baked goods [32]. The pyrazine in baked bread is mainly produced via the Maillard reaction, and the existence of the ammonium salt can promote the formation of pyrazine substances and improve the baked flavor characteristics of bread [33]. The OAV is commonly used to characterize the contribution of a compound to the aroma of a food system [34,35]. Using 2-octanol as internal standard, the types and contents of pyrazine compounds in bread samples with different ammonium salts were detected, and then the OAV was calculated using the content and threshold of each pyrazine. The total OAV of the pyrazine were obtained by adding them together, as shown in Table 3.

The pyrazine compounds obtained by adding varying types and amounts of ammonium salts to bread samples were distinct. 8 types of pyrazine substances were mainly detected when diammonium hydrogen phosphate was used in bread with a high level, which produced a greater number of pyrazines than any other groups. The more the used amount, the greater the total OAV of the resulting pyrazine substance with the same amount of added ammonium salt. Moreover, the total OAV generated in the bread samples with diammonium hydrogen phosphate at the three levels was the largest, especially the "medium" and "high" concentrations. The total OAV of the bread samples with a "high" content of added diammonium hydrogen phosphate reaches 39.86, which had the most obvious contribution to bread flavor. At the same supplemental level, the total OAV of the pyrazines produced in the bread sample with ammonium bicarbonate was lower, and the total OAV of the pyrazines produced in the bread sample with reducing sugar to produce pyrazine substances, which is also consistent with the rule of DOF formation between ammonium salts and reducing sugar. The addition of diammonium hydrogen phosphate can effectively improve the production of pyrazine flavor substances in bread and has a vital reference value for the preparation of bread with both functional and special baked flavor characteristics.

4. Conclusion

Three ammonium salts, diammonium hydrogen phosphate, ammonium bicarbonate, and ammonium carbonate, were used in bread-making as the additive. The color, texture, deoxyfructosazine, and characteristic pyrazine flavor substance, which were closely related to the quality of bread, were investigated. The texture of the bread samples with different ammonium salts were not significantly different, and compared to the control bread without the addition of ammonium salt, the hardness and viscosity increased. However, due to the Maillard reaction, their color was darker than that of the control samples, especially the samples with added diammonium hydrogen phosphate, whose crust was less bright and more red and yellow. The bread samples with ammonium salts produced abundant pyrazine compounds, including the deoxyfructosazine of the functional substances and some volatile flavor characteristics of pyrazine, which were caused by the Maillard reaction between the ammonium salts and reducing sugar in bread preparation. For deoxyfructosazine, when diammonium hydrogen phosphate was added, the deoxyfructosazine produced was higher than that when the other two ammonium salts were used at the same level. For flavor pyrazine, at the same supplemental level, the total value of odor activity of the pyrazine produced in the bread samples with diammonium hydrogen phosphate was the largest, that is, the contribution to the flavor characteristics of the bread was greater. The value of odor activity increased significantly with the increase in the usage of the ammonium salt. These rules provided a reference for the preparation of bread with both functionality and favorable quality, and also, provided a research basis for the application of ammonium salts in bread and other baked goods.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Ruiqi Sun: Writing – original draft, Investigation, Formal analysis, Data curation. Jiaqi Zheng: Project administration. Mengdi Niu: Resources, Investigation. Jun Wang: Writing – review & editing, Supervision, Conceptualization.

Table 3 Pyrazine substances of bread samples with different types and amounts of ammonium salts (n = 3).

Species	Level	Pyrazine species								Total
		2- methylpyrazine	2,6- dimethylpyrazine	2- ethylpyrazine	2,3- dimethylpyrazine	2-ethyl-6- methyl pyrazine	2,3,5- trimethylpyrazine	2,6- diethylpyrazine	tetramethylpyrazine	OAV
Diammonium	High	0.017	0.238	0.028	0.035	1.058	22.904	15.567	0.010	39.857
hydrogen	Medium	0.005	0.011	ND	0.011	ND	9.000	9.517	ND	18.545
phosphate	Low	0.010	ND	0.013	ND	ND	6.498	ND	ND	6.518
Ammonium	High	0.079	1.070	ND	0.025	ND	13.665	ND	ND	14.840
bicarbonate Medi Low	Medium	0.016	0.329	ND	ND	ND	5.530	ND	ND	5.875
	Low	0.084	0.713	ND	0.017	ND	4.609	ND	ND	5.423
Ammonium	High	0.179	1.627	ND	0.036	ND	4.626	ND	ND	6.468
carbonate	Medium	0.022	0.381	0.006	ND	ND	3.939	ND	ND	4.347
	Low	0.029	0.366	ND	ND	ND	1.344	ND	ND	1.738

ND: Not detected.

8

Declaration of competing interest

The authors declare no conflict of interest.

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