

Article

Effects of Nitrogen Fertilizer on the Endosperm Composition and Eating Quality of Rice Varieties with Different Protein Components

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Abstract: Nitrogen fertilizer affects rice endosperm protein, which in turn affects rice taste. However, study of nitrogen regulation of protein components is not sufficient. To clarify the effects of nitrogen fertilizer on rice protein components and related traits, we used two high albumin content and two low glutelin content rice varieties as test materials and analyzed the relationship between protein components and rice eating quality. The results showed that nitrogen application significantly affected prolamine and glutelin contents; moreover, a relationship was observed between variety and fertilization level. The protein components of the low glutelin content rice varieties were sensitive to nitrogen treatment; the albumin and globulin contents increased, whereas the prolamine and glutelin contents decreased following treatment with medium level nitrogen. Nitrogen treatment also significantly affected the apparent amylose content (AAC) of varieties except Yinguang. The eating characteristics of the high albumin content varieties differed significantly among nitrogen treatments, and the two varieties tasted better following treatment with high concentrations of nitrogen. In conclusion, the short chain of amylopectin in the endosperm had a greater direct effect on taste, but the effect was opposite in the two varieties. Furthermore, globulin was found to affect the taste of low glutelin content varieties.

Keywords: japonica rice; nitrogen fertilizer; protein components; starch components; eating characteristics



Citation: Ma, Z.; Zhu, Z.; Song, W.; Luo, D.; Cheng, H.; Wang, X.; Lyu, W. Effects of Nitrogen Fertilizer on the Endosperm Composition and Eating Quality of Rice Varieties with Different Protein Components. *Agronomy* **2024**, *14*, 469. <https://doi.org/10.3390/agronomy14030469>

Academic Editor: Yulong Ren

Received: 24 January 2024

Revised: 20 February 2024

Accepted: 26 February 2024

Published: 27 February 2024



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1. Introduction

Rice (*Oryza sativa* L.) is one of the world's most important food crops, with a planting area of 162.05 million hectares, of which China's rice planting area accounts for 18.5% of the world, with a total output of 210 million tons. With the improvement in living standards, the demand for rice has gradually shifted from yield to quality, with nutritional and taste qualities being the most important traits for consumers. Except for starch, protein is the most important endosperm component in rice, and its type and content are important factors for evaluating the nutrition and taste quality of rice.

Most studies on the effect of rice protein on cooking taste quality have mainly focused on protein content, with few on protein composition and form. High protein content inhibits water absorption, expansion, and gelatinization of starch grains. When the protein content of rice exceeds 7%, it exhibits poor taste [1,2]. However, some researchers believe that the taste quality mainly depends on the rice variety, and a high protein content does not necessarily reduce the taste value [3]. Therefore, protein content is not linearly correlated with taste.

Storage proteins [4] account for the highest proportion of rice protein and include albumin, globulin, prolamine, and glutelin. Glutelin accounts for the highest proportion

(approximately 80%) of the total endosperm protein, followed by prolamine [5], globulin, and albumin [6]. Furthermore, albumin has high lysine and threonine contents, which are important factors affecting the nutritional quality of rice. Protein plays a key role in the formation of rice chalkiness [7,8], which is mainly caused by differences in globulin and albumin accumulation. Prolamine and glutelin are unsuitable for the growth of good-tasting rice. Furthermore, prolamine can hinder the development of the starch mesh structure and is mostly responsible for the reduction in taste quality. In addition, both glutelin and globulin can reduce the adhesion of starch gel in a concentration-dependent manner [9].

Genetic factors and cultivation measures affect rice quality. Regarding cultivation measures, the effect of nitrogen application on rice quality has been the most studied [10]. Most studies have shown that an appropriate amount of nitrogen fertilizer can improve the milling quality of rice [11], promote starch accumulation in rice, and reduce chalkiness grain rate and chalkiness, thereby improving the appearance quality. Furthermore, nitrogen application reduced amylose content, increased protein content, and decreased taste value [12].

In conclusion, protein content and composition are important factors contributing to rice nutrition and taste quality and significantly affect other rice qualities. At present, studies on the effect of protein on rice quality are insufficient, and there is no consensus on the effect of nitrogen fertilizer on the content of different protein components. Therefore, in the present study, we selected typical varieties with high albumin and low glutelin contents, performed nitrogen fertilizer control during grout filling, and explored the changes in protein and starch properties and their relationship with eating taste to clarify the differences in protein and starch components among varieties and their effects on quality. It provides a theoretical basis for further improving the taste quality of rice by regulating endosperm composition with fertilizer.

2. Materials and Methods

2.1. Test Materials

In this study, according to the specificity of protein components, two high albumin content rice varieties, Jiyang 108 and Yinguang, and two low glutelin content rice varieties, Jinongda 815 and Xinnongjing 8, were selected from the japonica rice varieties in China.

2.2. Field Cultivation Management

The experiment was conducted in the Nandi paddy field test field of Shenyang Agricultural University in 2022, and the rice was sown on April 18 and transplanted on May 19. The basic conditions of the soil of the field were as follows: available N, 65.70 mg kg⁻¹; available P, 13.59 mg kg⁻¹; available K, 120.40 mg kg⁻¹; organic matter, 25.63 mg kg⁻¹; soil pH, 5.53; conductivity (5:1), 274.70; and conductivity (2.5:1), 294.70. A two-factor randomized block design was used. Three nitrogen fertilizer treatments were used: (1) Low N: 0.00476 kg·m⁻² nitrogen fertilizer, (2) Medium N: 0.00595 kg·m⁻² nitrogen fertilizer, and (3) High N: 0.00714 kg·m⁻² nitrogen fertilizer. The proportions of nitrogen fertilizer in the three treatments were 16.68%, 20.01%, and 23.09%, respectively. The base fertilizer and topdressing treatment of the three treatments were the same, i.e., the base fertilizer was a special rice fertilizer with an N–P–K content of 24–10–14, and the nitrogen content was 0.0149 kg·m⁻². The green fertilizer was applied with ammonium sulfate 18 days after transplantation, with a nitrogen content of 0.00236 kg·m⁻², and the tiller-urea was applied 25 days after transplantation, with a nitrogen content of 0.00648 kg·m⁻². Each treatment was repeated thrice, with a length of 1.5 m and four rows, and the distance between rows and plants was 30 cm × 13.3 cm.

2.3. Measurement of the Characteristics

2.3.1. Rapid Viscosity Analysis

A rapid viscosity analyzer (TecMaster, Newport Scientific, Sydney, Australia) and TCW3 software were used for the analysis of gelatinization characteristics. The relative deviation between the measured and mean values of each group of samples was <5%.

2.3.2. Determination of the Protein Components

Protein components were determined using a modified Bradford method [13] protein concentration determination kit (product No.: C503041) from Biochem, Inc. (Shanghai, China), and was determined using Thermo Fisher 3001 (Shanghai, China).

2.3.3. Configuration of the Standard Protein Solution

First, 12 1.5-mL centrifuge tubes were used, and one repeat for each standard product was set. Then, 1 mg/mL of bovine serum albumin (BSA) standard protein solution (0, 5, 10, 15, 20, 25, and 30 μ L) was added to each tube and mixed quickly, then fill with distilled water to 1000 μ L. After reaction at 25–30 $^{\circ}$ C for 10 min, the A_{595} value of each tube was measured using the spectrophotometer with No. 0 tube serving as the blank control. The average value of each A_{595} tube was taken as the ordinate, and the corresponding protein concentration was taken as the horizontal coordinate. The standard curve was drawn in Microsoft Excel.

2.3.4. Determination of the Albumin Content

First, 0.1 g of rice flour was weighed into a 1.5-mL centrifuge tube. Then, 1 mL of distilled water was added, shaken at room temperature for extraction for 2 h, and centrifuged at 10,000 rpm for 10 min. Next, the supernatant was poured into a 5-mL test tube. The extraction procedure was repeated thrice before the extraction solutions were combined. Then, 20 μ L of the resultant solution was absorbed and dropped into the enzyme label plate. Subsequently, 200 μ L of Bradford working liquid was added, quickly mixed at room temperature (25–30 $^{\circ}$ C), and reacted for 5 min. The A_{595} value of each hole was measured using an enzyme label instrument. A standard solution of BSA was used as the standard curve.

2.3.5. Determination of the Globulin Content

First, 1 mL of 5% sodium chloride solution was added to the rice flour precipitate containing the extracted albumin. The extraction and determination processes were the same as those for albumin.

2.3.6. Determination of the Prolamine Content

First, 1 mL of 70% ethanol solution was added to the rice flour precipitate containing the extracted globulin. The extraction and determination process is the same as that for albumin.

2.3.7. Determination of the Glutelin Content

First, 1 mL of 0.2% sodium hydroxide solution was added to the rice flour precipitate containing the extracted prolamine, shaken, and then extracted on the shaking table for two hours. Then, the supernatant was centrifuged at 12,000 $\text{r}\cdot\text{min}^{-1}$ for 10 min, extracted thrice, and then combined.

2.3.8. Determination of the Amylose Content

Refer to “NY/T2639-2014 spectrophotometric method for the determination of amylose in rice”, 620 nm colorimetric determination method.

2.3.9. Determination of the Amylopectin Content

Referring to the method of Nakamura et al. [14], the peak wavelength of starch iodine adsorption λ_{\max} was obtained using an ultraviolet spectrophotometer at 400–900 nm, and λ_{\max} was the corresponding absorbance of λ_{\max} . F_2 is the area from 400 nm to λ_{\max} , and $\text{New}\lambda_{\max}$ is the value minus the influence of glutinous rice. F_a , $F_{b_{1+2}}$, and F_{b_3} were calculated according to the following formula:

$$\begin{aligned}\text{New}\lambda_{\max} &= \frac{73.307 \times A\lambda_{\max} + 0.111 \times \lambda_{\max} - 73.016}{\lambda_{\max} \text{ of Test sample} - \lambda_{\max} \text{ of Glutinous rice}} \\ F_a &= -11.59 \times F_2 - 10.92 \times \text{New}\lambda_{\max} + 34.429 \\ F_{b_3} &= 44.691 \times A\lambda_{\max} - 0.774 \\ F_{b_{1+2}} &= 1 - (F_a + F_{b_3})\end{aligned}$$

2.4. Data Analysis

The DPS 15.10 data processing system was used to analysis of variance, multiple comparison and path analysis. GraphPad Prism 8.2.1 mapping software was used for drawing.

3. Results and Discussion

3.1. Response of the Endosperm Components of the Rice Varieties with Different Protein Components to Nitrogen Fertilizer Application

The cooking and eating quality of rice mainly depends on its starch properties and protein components. Therefore, nitrogen fertilizer application can improve the eating quality of rice mainly by affecting these two main physicochemical indexes [15,16]. In this study, nitrogen application significantly affected the amylose content, and a relationship between variety and fertilization level was observed (Table S1). The AAC of the high albumin content varieties increased with an increase in nitrogen application, whereas the AAC of the low glutelin content varieties fluctuated with an increase in nitrogen application, possibly due to the competitive relationship between the protein and amylose contents of rice (Figure 1). Previous studies have shown that nitrogen can change the content and structure of amylopectin in rice as well as increase the proportion of short chains; however, it is unsuitable for the formation of long chains [17]. The F_a and F_{b_3} of Xinnongjing8 and Yinguang in this study increased with an increase in nitrogen application. For potatoes, studies have shown that the amount of nitrogen fertilizer application significantly affects the expression of the starch synthetase gene SSIII; reasonable nitrogen fertilizer application can increase SSIII expression level [18]. Therefore, in this study, did the application of rice panicle fertilizer increase the expression level of SSIII and promote the synthesis of long-chain amylopectin? To answer the above question, further investigation is needed. In general, nitrogen application significantly affected the branch structure of amylopectin of the high albumin content variety Yinguang, whereas the AAC of the other three varieties differed significantly in response to nitrogen fertilizer.

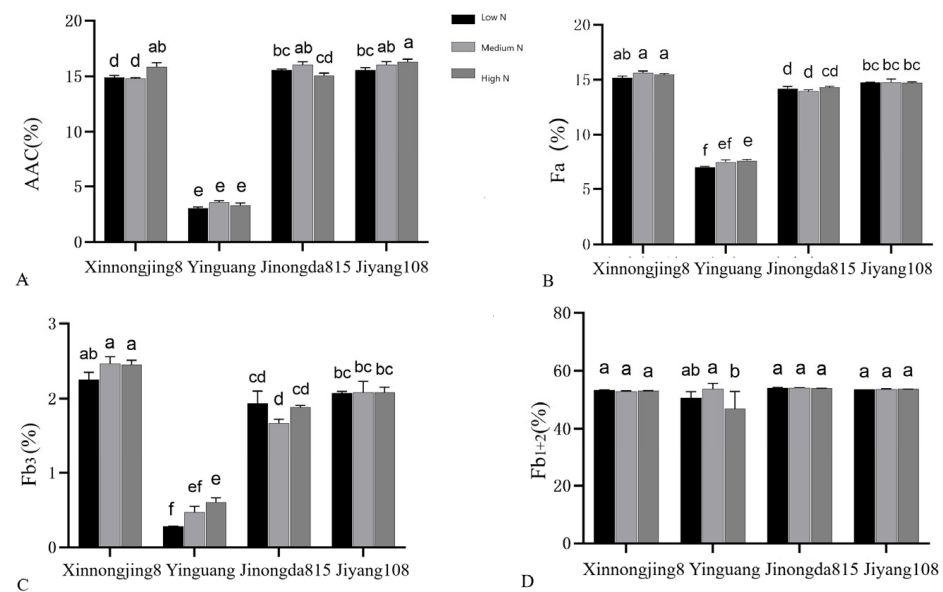


Figure 1. Effect of nitrogen fertilizer on starch composition of different rice varieties. (A): Differences of AAC (apparent amylose content) in four varieties with different nitrogen application levels, (B): Differences of Fa (short chain of amylopectin) in four varieties with different nitrogen application levels, (C): Differences of Fb₃ (long chain of amylopectin) in four varieties with different nitrogen application levels, (D): Differences of Fb₁₊₂ (medium chain of amylopectin) in four varieties with different nitrogen application levels. Note: The different letters indicate that the difference is significant at 0.05 level.

Many scholars have relatively consistent views on the effect of nitrogen fertilizer on the protein content of rice. Nitrogen fertilizer significantly affects the protein content of rice, but it had marginal utility [19]. However, the effect is marginal, and nitrogen fertilizer application at the booting stage or increasing the application ratio at the ear stage is the most beneficial for protein accumulation. Studies have shown that after nitrogen application (Figure 2), changes in prolamine and glutelin contents are more prominent, albumin and globulin contents are less affected, and the effects of nitrogen on the same protein component of different rice varieties also differ [20]. This study also confirmed that nitrogen application significantly affected prolamine and glutelin contents, and the variance between fertilization treatment and the interaction of variety \times fertilization level was significant (Table S2). From the perspective of variety type, nitrogen application insignificantly affected the high albumin-containing varieties, while the protein components of the low glutelin-containing varieties were sensitive to nitrogen application. Medium application of nitrogen increased albumin and globulin contents and decreased prolamine and glutelin contents. Additionally, nitrogen application significantly affected the proportion of protein components in Xinnongjing 8 and the proportion of prolamine content in Yinguang and Jinongda 815. Therefore, it can be further confirmed that the effects of nitrogen fertilizer on protein content and composition vary with variety. Nitrogen fertilizer treatment can change the protein content and composition of rice as a whole and improve the cooking and eating quality of rice, but it needs to be controlled according to the variety characteristics. In addition, the distribution of the four proteins in rice grains was also different. Gluten gradually increased from the outer to the inner endosperm, globulin and albumin mainly existed in the outer part of rice (i.e., aleurone layer), and promaline is uniformly distributed throughout the whole region of the rice [21]. Therefore, from the point of grain development, gluten accumulates first, while globulin and albumin accumulate later. According to the accumulation time characteristics of different protein components, can we adjust the application period of ear granule fertilizer to regulate the content of a certain protein component in a targeted way? It is worth further study.

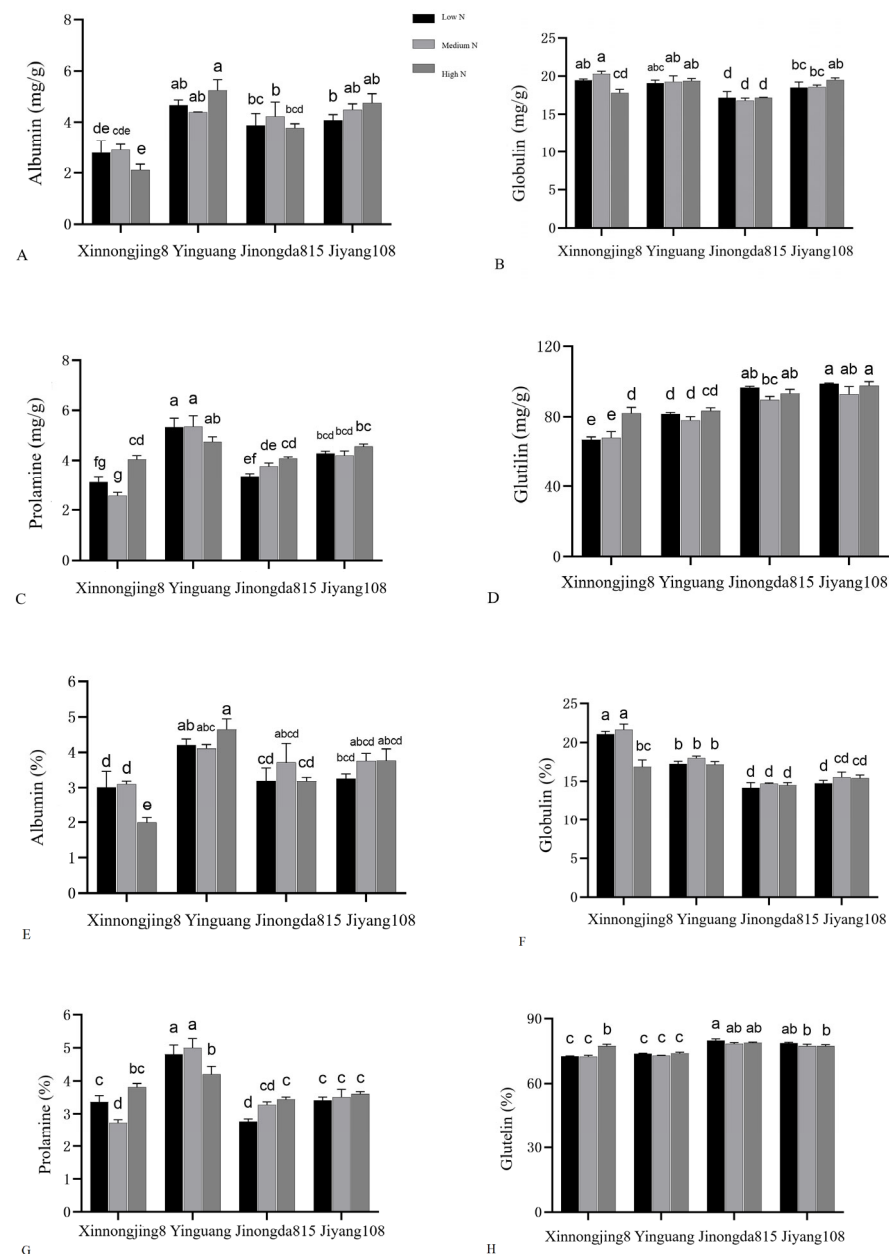


Figure 2. Effects of nitrogen fertilizer on protein composition and proportion of different rice varieties. (A): Differences of albumin content in four varieties with different nitrogen application levels, (B): Differences of globulin content in four varieties with different nitrogen application levels, (C): Differences of prolamine content in four varieties with different nitrogen application levels, (D): Differences of glutelin content in four varieties with different nitrogen application levels, (E): Differences of albumin percentage in four varieties with different nitrogen application levels, (F): Differences of globulin percentage in four varieties with different nitrogen application levels, (G): Differences of prolamine percentage in four varieties with different nitrogen application levels, (H): Differences of glutelin percentage in four varieties with different nitrogen application levels. Note: The different letters indicate that the difference is significant at 0.05 level.

3.2. Response of Rice Varieties with Different Protein Components to Nitrogen Fertilizer Application in Terms of Eating Quality

The RVA spectrum of rice starch is an important index for evaluating rice quality, which is closely related to cooking and eating quality. The RVA starch spectrum parameters of rice are significantly affected by the nitrogen supply level, and the performance differs among varieties, which is related to the experimental varieties, cultivation environment,

and experimental conditions. RVA characteristic values of the different varieties differed significantly, indicating that variety significantly influenced RVA traits. However, previous studies have found that the coefficient of variation between the trough viscosity and the final viscosity is larger [22], indicating that nitrogen fertilizer significantly affected the two RVA eigenvalues. The results of this study also showed that the nitrogen application level significantly affected the trough and final viscosities (Figure 3, Table S3). Generally, the starch viscosity of rice tends to deteriorate with increasing nitrogen fertilizer application. However, in this study, low protein varieties showed a higher viscosity value under high nitrogen (N3) treatment. For high albumin-containing varieties, RVA of Yinguang was not sensitive to fertilization, while the RVA (except backdown) of Jiyang 108 was lower under low nitrogen treatment and higher under high nitrogen level. It can be seen that high nitrogen treatments positively affected the RVA value of the two varieties, but it rarely has a significant impact, which is related to the small fertilizer horizontal gradient set in this study and the nitrogen fertilizer does not reach the critical value.

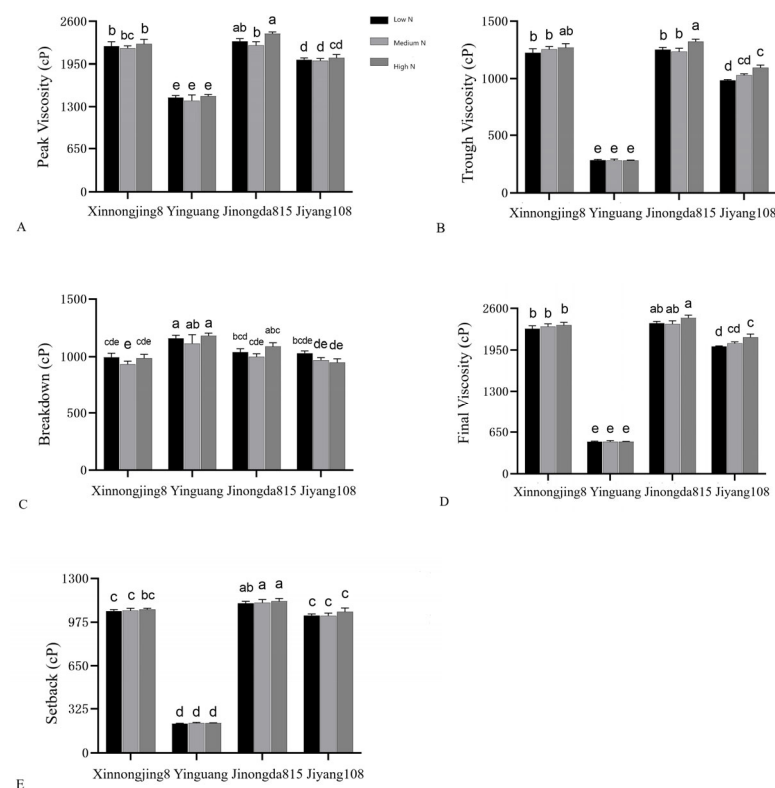


Figure 3. Effect of nitrogen fertilizer on RVA characteristic values of different rice varieties. (A): Differences of peak viscosity in four varieties with different nitrogen application levels, (B): Differences of trough viscosity in four varieties with different nitrogen application levels, (C): Differences of breakdown in four varieties with different nitrogen application levels, (D): Differences of final viscosity in four varieties with different nitrogen application levels, (E): Differences of setback in four varieties with different nitrogen application levels. Note: The different letters indicate that the difference is significant at 0.05 level.

The taste value of rice is a comprehensive feeling, including vision, taste, and smell, and the taste quality of rice is an important part of the rice quality. The results the present study showed that varieties and fertilization levels significantly affected food taste value, appearance, hardness, viscosity, and balance (Figure 4, Table S4). Variety and fertilization level significantly influenced hardness, and a relationship was suspected between the two. This indicated that nitrogen fertilizer application greatly affects food quality. Several studies have concluded that late nitrogen fertilizer application adversely affects rice quality [23]. With increasing nitrogen application, most varieties show an increase in rice hardness

and a decrease in appearance, viscosity, balance, and taste value, especially when the nitrogen content is high. The eating characteristics of high albumin content varieties differed significantly among nitrogen treatments. Under high nitrogen application, the hardness of these varieties was the lowest, and the other taste traits were the highest. Low protein varieties were insensitive to fertilization treatment. Regarding the relationship between nitrogen application rate and rice's excellent taste, some studies have suggested that the optimal nitrogen application rate for japonica rice's taste was 0.0240–0.02625 kg·m⁻² [24]. However, the high nitrogen setting rate in this study did not reach this range. Moreover, because high nitrogen application promoted the content of short-chain amylopectin, albumin, and globulin of high albumin content varieties, it was concluded that high nitrogen application was conducive to taste formation of these varieties. Because food taste is also related to fat and mineral content, the reasons for the difference in food taste value with nitrogen response will be further studied and analyzed from these aspects.

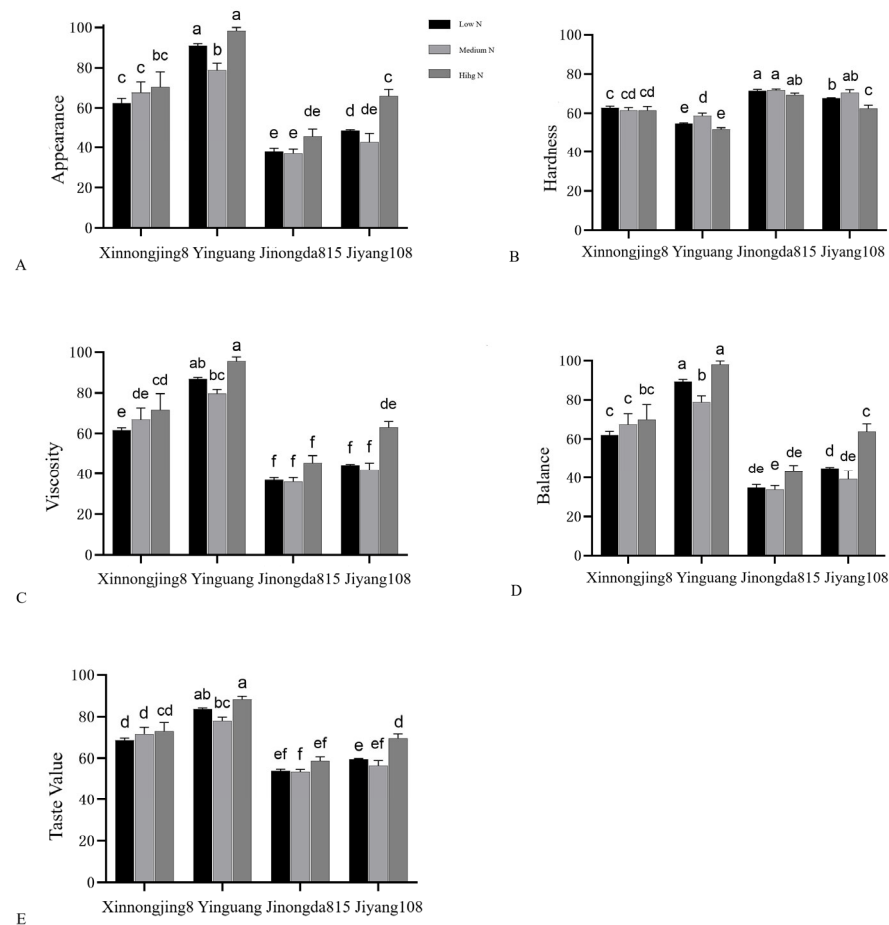


Figure 4. Effects of nitrogen fertilizer on eating quality of different rice varieties. (A): Differences of appearance in four varieties with different nitrogen application levels, (B): Differences of hardness in four varieties with different nitrogen application levels, (C): Differences of viscosity in four varieties with different nitrogen application levels, (D): Differences of balance in four varieties with different nitrogen application levels, (E): Differences of taste value in four varieties with different nitrogen application levels. Note: The different letters indicate that the difference is significant at 0.05 level.

3.3. Path Analysis of the Relationship between Endosperm Components and Food Quality

Although personal preferences influence the evaluation of rice taste, the core factor is the viscoelasticity of rice. Good taste of rice is characterized by strong viscosity and elasticity and moderate hardness when chewed; these characteristics are related to the endosperm composition. Within a certain range, the lower the AAC with low water absorption and swelling, the greater the viscosity of the rice, the softer the rice, and the better its taste.

In general, the AAC of rice with better taste is <18% [25]. However, taste among similar AAC varieties has been reported to differ and mainly affected by amylopectin. In the amylopectin side chain, the greater the proportion of short branches to long branches, the softer the rice [26], whereas the lower or higher the proportion of medium chains, the harder the rice [19] and more difficult to cook [27]. A previous study also showed that a high long-chain Fb_3 ratio of amylopectin increases the hardness of rice, and high Fa and Fb_{1+2} contents and Fa/Fb_3 ratio increase the viscosity of rice [28]. For japonica rice varieties with slight difference in amylose content, protein content is the main factor affecting the taste quality of rice. However, the relationship between protein content and food taste value is not fixed, and the food taste value of rice usually increases with a decrease in protein content. When the protein content decreases to a certain level, the food taste value of rice tends to decrease. Through the detection of rice protein components, it was found that albumin content in indica rice had a significant negative correlation with food taste value, whereas albumin content in japonica rice had a significant positive correlation or an insignificant negative correlation with food taste value. A high content of prolamine negatively affects food taste value, whereas glutelin can form various hydrogen bonds with water, and the water-holding capacity and hydration degree of starch are weakened when glutelin is increased [9]. Therefore, glutelin also negatively affects food taste value, but it can improve the food taste quality of rice. Because several varieties were selected in previous studies, it is evident that the effects of protein content and components on food taste value may vary with variety. In this study, the path analysis of starch, protein, and taste value of the two varieties revealed that the direct effect of amylopectin Fa , Fb_3 , and AAC in high albumin varieties were the largest (Tables 1 and 2). The direct coefficients of all endosperm components, except Fa , were less than the sum of indirect coefficients, and mainly through the indirect effects of Fa and Fb_3 , indicating that the short-chain ratio of amylopectin significantly affected the taste value of high albumin-containing varieties. In the low glutelin-containing varieties, the direct effect of Fa and Fb_{1+2} on taste value was the largest and positive. For indirect effects, globulin content and Fa were the main factors. The results show that the direct effect of Fa is very significant in the two varieties, but the effect is the opposite. Higher Fa content is suitable for the performance of the low glutelin-containing varieties but unsuitable for the high albumin-containing varieties, further confirming that its effect on taste varies according to variety, which also confirms that protein content determines the interaction between protein and starch [29] as well as affects the formation of hydrogen bonds and gel networks within starch molecules [30], thereby affecting taste quality. However, further studies are needed to determine whether the difference in protein content and types of rice changes rice taste quality and whether it is caused by the direct effect of protein or the indirect effect of the synthesis of starch structure or starch gelatinization. In addition, due to the different distribution of protein components in rice grains, do the protein components at different layers of endosperm have positive or negative effects on taste value? Is there a difference influence of protein components on taste among different layers? It needs further study and discussion.

Table 1. Path analysis of endosperm composition and taste value of high albumin content varieties.

Endosperm Component	Direct Coefficients	Sum of Indirect Coefficients	Through the X1	Through the X2	Through the X3	Through the X4	Through the X5	Through the X6	Through the X7	Through the X8	Determination Coefficient
AAC (X1)	0.61	−1.50		−3.21	1.74	0.00	−0.01	−0.02	0.17	−0.17	R ² = 0.921
Fa (X2)	−3.25	2.37	0.60		1.81	0.00	−0.01	−0.02	0.16	−0.17	
Fb ₃ (X3)	1.85	−2.67	0.58	−3.19		−0.01	−0.01	−0.02	0.15	−0.17	
Fb ₁₊₂ (X4)	−0.03	−0.07	0.04	−0.51	0.49		0.00	−0.02	−0.04	−0.01	
Albumin (X5)	0.04	0.34	−0.14	0.71	−0.29	0.00		0.03	0.02	0.02	
Globulin (X6)	0.10	0.11	−0.13	0.75	−0.46	0.01	0.01		−0.10	0.02	
Prolamine (X7)	−0.25	0.76	−0.41	2.13	−1.11	0.00	0.00	0.04		0.11	
Glutelin (X8)	−0.19	−0.59	0.53	−2.87	1.63	0.00	0.00	−0.01	0.15		

Table 2. Path analysis of endosperm composition and taste value of low glutelin content varieties.

Endosperm Component	Direct Coefficients	Sum of Indirect Coefficients	Through the X1	Through the X2	Through the X3	Through the X4	Through the X5	Through the X6	Through the X7	Through the X8	Determination Coefficient
AAC (X1)	−0.08	−0.02		−1.50	0.30	1.01	−0.01	0.41	−0.07	−0.17	R ² = 0.934
Fa (X2)	4.79	−3.97	0.03		−1.10	−2.86	0.06	−0.45	0.06	0.28	
Fb ₃ (X3)	−1.12	1.94	0.02	4.68		−2.71	0.06	−0.43	0.06	0.25	
Fb ₁₊₂ (X4)	2.90	−3.67	−0.03	−4.73	1.05		−0.06	0.46	−0.06	−0.30	
Albumin (X5)	−0.08	−0.65	−0.01	−3.75	0.90	2.15		0.28	−0.02	−0.20	
Globulin (X6)	−0.66	1.02	0.05	3.26	−0.72	−2.01	0.03		0.10	0.29	
Prolamine (X7)	−0.16	−0.22	−0.03	−1.80	0.39	1.07	−0.01	0.41		−0.24	
Glutelin (X8)	−0.37	−0.22	−0.04	−3.70	0.77	2.37	−0.04	0.53	−0.11		

4. Conclusions

Nitrogen application significantly affected the content of prolamine and glutelin; this effect was obvious in the low glutelin content varieties. Nitrogen fertilizer treatment increased the proportion of short chain (Fa) and long chain (Fb₃) of amylopectin in Xinnongjing8 and Yinguang. The AAC of the high albumin content variety Jiyang108 showed an increasing trend with an increase in nitrogen fertilizer application, whereas the AAC of the low glutelin content varieties showed a fluctuation. High nitrogen levels promoted the RVA viscosity and taste value of two type varieties, and had a significant effect on the taste value of the high albumin content variety. Among the endosperm components, starch had a greater direct effect on taste, but the effect was the opposite in the high albumin content and low glutelin content varieties, so were the albumin and globulin.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14030469/s1>, Table S1: ANOVA of the effect of nitrogen fertilizer regulation on starch characteristics; Table S2: ANOVA of the effect of nitrogen fertilizer regulation on protein components; Table S3: ANOVA of the effect of nitrogen fertilizer regulation on RVA characteristic; Table S4: ANOVA of the effect of nitrogen fertilizer regulation on taste traits.

Author Contributions: Conceptualization, W.L. and X.W.; methodology, W.L.; software, H.C.; validation, W.L., Z.M. and Z.Z.; formal analysis, H.C.; investigation, Z.Z., W.S. and D.L.; resources, W.L.; data curation, Z.M.; writing—original draft preparation, Z.M.; writing—review and editing, W.L. and Z.M.; visualization, H.C.; supervision, W.L.; project administration, W.L.; funding acquisition, Z.M. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the National Natural Science Foundation of China (32201892), Liaoning Province Department of Education Project (LJKMZ20221016), Inner Mongolia important research and development plan (2023YFDZ0041), and Ministry of Agriculture Accurate Identification Project (19230583).

Data Availability Statement: Data are contained within the article.

Acknowledgments: The test work of this experiment was completed in the Test Center of the Agricultural College of Shenyang Agricultural University.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Lim, S.T.; Lee, J.H.; Shin, D.H.S.; Lim, H.S. Comparison of protein extraction solutions for rice starch isolation and effects of residual protein content on starch pasting properties. *Starch-Stärke* **1999**, *51*, 120–125. [\[CrossRef\]](#)
2. Lyon, B.G.; Champagne, E.T.; Vinyard, B.T.; Windham, W.R. Sensory and instrumental relationships of texture of cooked rice from selected cultivars and postharvest handling practices. *Cereal Chem.* **2000**, *77*, 64–69. [\[CrossRef\]](#)
3. Xiang, H.Y.; Tang, Q.Y.; Huang, Y.X. The relativity of rice grain quality characteristics—I Relations between eating quality and other grain quality characteristics of indica non-waxy rice. *J. Hunan Agric. College* **1990**, *4*, 325–330. (In Chinese with English Abstract)
4. Lu, K.; Zhao, Q.Y.; Zhou, L.H.; Zhao, C.F.; Zhang, Y.D.; Wang, C.L. Research progress on the relationship between rice protein content and eating quality and the influence factors. *J. Agric. Sci.* **2020**, *36*, 239–245. (In Chinese with English Abstract)
5. Kubota, M.; Saito, Y.; Masumura, T.; Kumagai, T.; Watanabe, R.; Fujimura, S.; Kaduwak, M. Improvement in the in vivo digestibility of rice protein by alkali extraction is due to structural changes in prolamin/protein body-I particle. *Biosci. Biotechnol. Biochem.* **2010**, *74*, 614–619. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Wang, Z.C.; Yao, H.Y. Advances in molecular characterization of rice protein. *Grain Oil* **2003**, *10*, 3–6. (In Chinese with English Abstract)
7. Xi, M.; Wu, W.G.; Xu, Y.Z.; Zhou, Y.J.; Chen, G.; Ji, Y.L.; Sun, X.Y. Grain chalkiness traits is affected by the synthesis and dynamic accumulation of the storage protein in rice. *J. Sci. Food Agric.* **2021**, *101*, 6125–6133. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Hiroshi, W.; Yuto, H.; Yayoi, O.; Hiroshi, N.; Taiken, N.; Rosa, E.B.; Satoshi, M.; Kenzo, H.; Fukuyo, T.; Hiroshi, N. Multiple strategies for heat adaptation in the rice endosperm. *J. Exp. Bot.* **2019**, *70*, 1299–1311.
9. Baxter, G.; Blanchard, C.; Zhao, J. Effects of glutelin and globulin on the physicochemical properties of rice starch and flour. *J. Cereal Sci.* **2014**, *60*, 414–420. [\[CrossRef\]](#)
10. Alcantara, J.M.; Cassman, K.G.; Consuelo, M.; Bienvenido, O.; Samue, I.P. Effects of late nitrogen fertilizer application on head rice yield, protein content, and grain quality of rice. *Cereal Chem.* **1996**, *73*, 556–560.

11. Chen, C.; Zeng, Y.J.; Wang, Q.; Tan, X.M.; Shang, Q.Y.; Zeng, Y.H.; Shi, Q.H.; Jin, X. Effects of nitrogen application regime on japonica rice yield and quality of the late rice in the double rice system in southern China. *J. Plant Nutr. Ferti.* **2018**, *24*, 1386–1395. (In Chinese with English Abstract)
12. Jian, L.J. Effect of nitrogen application on yield and quality of japonica rice. *Anhui Agric. Sci. Bul.* **2020**, *26*, 37–39. (In Chinese with English Abstract)
13. Shi, L.; Zhang, X.Y.; Sun, H.Y.; Cao, X.M.; Liu, J.; Zhang, Z.J. Relationship of grain protein content with cooking and eating quality as affected by nitrogen fertilizer at late growth stage for different types of rice varieties. *Chin. J. Rice Sci.* **2019**, *33*, 541–552. (In Chinese with English Abstract)
14. Nakamura, S.; Satoh, H.; Ohtsubo, K. Development of formulae for estimating amylose content, amylopectin chain length distribution, and resistant starch content based on the iodine absorption curve of rice starch. *Biosci. Biotech. Biochem.* **2015**, *79*, 443–455. [[CrossRef](#)] [[PubMed](#)]
15. Yuan, S.; Su, Y.T.; Chen, P.P.; Yi, Z.X. Research progress and prospect of the effect of nitrogen application on rice quality. *Crop Res.* **2021**, *35*, 394–400. (In Chinese with English Abstract)
16. Wen, C.Y.; Xiong, Y.H.; Yao, X.Y.; Chen, C.L.; Hu, B.L.; Huang, Y.P.; Wu, Y.S. Effects of nitrogen application on yield, rice quality and processing characteristics in rice noodle-specific varieties. *Chin. J. Rice Sci.* **2020**, *34*, 574–585. (In Chinese with English Abstract)
17. Zhu, D.; Zhang, H.; Guo, B.; Xu, K.; Dai, Q.; Wei, C.; Huo, Z. Effects of nitrogen level on structure and physicochemical properties of rice starch. *Food Hydrocolloid* **2017**, *63*, 525–532. [[CrossRef](#)]
18. Marshall, J.; Sidebottom, C.; Debet, M.; Martin, C.; Smith, A.M.; Edwards, A. Identification of the major starch synthase in the soluble fraction of potato tubers. *Plant Cell* **1996**, *8*, 1121–1135.
19. Tang, J.; Tang, C.; Guo, B.W.; Zhang, C.X.; Zhang, Z.Z.; Wang, K.; Zhang, H.C.; Chen, H.; Sun, M.Z. Effects of nitrogen application on yield and rice quality of mechanical transplanting high quality late rice. *Acta Agron Sin.* **2020**, *46*, 117–130. (In Chinese with English Abstract) [[CrossRef](#)]
20. Li, H.Y.; Prakash, S.; Nicholson, T.M.; Melissa, A.; Gilbert, R.G. Instrumental measurement of cooked rice texture by dynamic rheological testing and its relation to the fine structure of rice starch. *Carbohydr. Polym.* **2016**, *146*, 253–263. [[CrossRef](#)]
21. Ning, H.; Liu, Z.; Wang, Q.; Lin, Z.; Chen, S.; Li, G.; Wang, S.; Ding, Y. Effect of nitrogen fertilizer application on grain phytic acid and protein concentrations in japonica rice and its variations with genotypes. *J. Cereal Sci.* **2009**, *50*, 49–55. [[CrossRef](#)]
22. Zhu, Z.; Zhang, Y.D.; Zhu, F.F.; Chen, T.; Zhao, Q.Y.; Yao, S.; Zhou, L.H.; Zhao, L.; Zhao, C.F.; Liang, W.H.; et al. Impact of nitrogen fertilizer management on physicochemical indices related to cooking and eating qualities of japonica rice cultivar Nanjing 505. *Jiangsu Agric. Sci.* **2021**, *49*, 84–89. (In Chinese with English Abstract)
23. Zhang, H.C.; Wang, X.Q.; Dai, Q.G.; Huo, Z.Y.; Ke, X.U. Effects of N-application rate on yield, quality and characters of nitrogen uptake of hybrid rice variety liangyoupeijiu. *Sci. Agric. Sin.* **2003**, *36*, 800–806. (In Chinese with English Abstract)
24. Zhang, Q. Researches on Characteristics of the Good-Taste and High-Yield Soft Japonica Rice and It's Corresponding Nitrogen Management in Taihu Lake Region. Master's Thesis, Yangzhou University, Yangzhou, China, 2021. (In Chinese).
25. Teng, B.; Zeng, R.; Wang, Y.; Liu, Z.; Zhang, Z.; Zhu, H.; Ding, X.; Lim, W.; Zhang, G. Detection of allelic variation at the Wx locus with single segment substitution lines in rice (*Oryza sativa* L.). *Mol. Breed.* **2012**, *30*, 583–595. [[CrossRef](#)]
26. Aoki, N.; Umemoto, T.; Okamoto, K.; Suzuki, Y.; Tanaka, J. Mutants that have shorter amylopectin chains are promising materials for slow-hardening rice bread. *J. Cereal Sci.* **2015**, *61*, 105–110. [[CrossRef](#)]
27. Gayin, J.; Abdel, A.E.; Manfu, L.J.; Bertoft, E. Unit and internal chain profile of African rice (*Oryza glaberrima*) amylopectin. *Carbohydr. Polym.* **2016**, *137*, 466–472. [[CrossRef](#)] [[PubMed](#)]
28. Ma, Z.H.; Cheng, H.T.; Nitta, Y.; Aoki, N.; Chen, Y.; Chen, H.X.; Ohsugi, R.; Lyu, W.Y. Differences in viscosity of superior and inferior spikelets of japonica rice with various percentages of apparent amylose content. *J. Agric. Food Chem.* **2017**, *65*, 4237–4246. [[CrossRef](#)] [[PubMed](#)]
29. Liu, K.L.; Zheng, J.B.; Chen, F.S. Relationships between degree of milling and loss of Vitamin B, minerals, and change in amino acid composition of brown rice. *LWT-Food Sci. Technol.* **2017**, *82*, 429–436. [[CrossRef](#)]
30. Li, Z.; Wang, L.; Chen, Z.; Yu, Q.; Feng, W. Impact of binding interaction characteristics on physicochemical, structural, and rheological properties of waxy rice flour. *Food Chem.* **2018**, *266*, 551–556. [[CrossRef](#)]

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