Effects of Different Application Periods and Methods of Fish Protein Peptide on Fruit Quality of 'Tieshanzha'

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Abstract [**Objectives**] This study adopted a three-factor three-level orthogonal design to explore the effects of different application periods and methods of fish protein peptide on the fruit quality of 'Tieshanzha'. [**Methods**] Factor A was set as the application period, with three levels: fruit-setting stage, core-hardening stage, and pre-coloring stage. Factor B was set as the application method, with three levels: root application, foliar spray, and root application + foliar spray. Factor C was set as the application concentration, with three levels: 0, 5 and 10 ml/L. [**Results**] Application period had an extremely significant effect on single fruit weight. Fertilization at the fruit-setting stage showed a single fruit weight as high as 13.36 g, which was 27.9% and 24% higher than those achieved by fertilization at the core-hardening stage and the pre-coloring stage, respectively. The factor that had the greatest impact on the internal quality of hawthorn fruit, specifically the Vc content, was application method. The optimal combination was foliar spray at the core-hardening stage with a concentration of 10 ml/L, which achieved the best fertilization effect. [**Conclusions**] This study provides a theoretical basis for improving fruit quality of 'Tieshanzha'.

Key words Hawthorn; Fish protein peptides; Fruit; Appearance; Quality

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Hawthorn (*Crataegus pinnatifidala* bunge), a deciduous tree species of the Rosaceae family, is native to China and exhibits strong stress resistance and wide adaptability[1]. *Crataegus* plants are widely distributed across Asia, Europe, North and Central America, and northern South America. China is one of the origin centers of *Crataegus* plants, with rich germplasm resources[2]. As a unique dual-purpose medicinal and edible resource in China, hawthorn fruit is rich in bioactive compounds such as flavonoids, organic acids, and polyphenols, making it valuable for fresh consumption, processing, and medicinal applications[3]. With the rapid development of the health industry, the market demand for high-quality hawthorn raw materials continues to grow[4]. However, targeted regulation of fruit quality has become a core bottleneck for industrial upgrading. In current cultivation practices, fertilization management predominantly relies on empirical single-mode approaches, lacking dynamic nutrient regulation strategies based on phenological characteristics. This results in prominent issues such as unstable commercial traits of fruit and inefficient accumulation of functional components.

Fish protein peptide fertilizer is an organic functional fertilizer derived from deep-sea fish protein through enzymatic hydrolysis[5]. Its core components include small molecular peptides, composite amino acids, and bioactive enzymes, which form a complex system after being compounded with trace elements and plant growth regulators sourced from deep-sea plants[6]. This fertilizer features a high organic matter content (≥45%), a scientifically balanced ratio of macronutrients, and a comprehensive spectrum of micronutrients, effectively meeting the nutritional needs of crops throughout their entire growth cycle. Studies have confirmed that its application significantly enhances chloroplast function and light energy conversion efficiency, specifically manifested by a 15% - 20% increase in the chlorophyll a/b ratio and elevated RuBisCO enzyme activity, thereby improving the translocation efficiency of photosynthetic products to grains.

In this study, 'Tieshanzha', a characteristic northern economic tree species, was selected as the test material to systematically investigate the effects of different application methods and concentrations of fish protein peptide fertilizer at various developmental stages throughout its annual growth cycle on fruit quality.

Materials and Methods

Experimental site

The experiment was conducted at Xinyuan Ziqi Family Farm in Xidaogou Village, Beiyingfang Town, Xinglong County, Chengde City, Hebei Province (117°29′ E, 40°35′ N). The altitude ranges from approximately 400 to 800 m. This area has an average annual temperature of 8 - 12 ℃, a frost-free period of 160 - 180 d, annual precipitation of about 600 - 700 mm, and annual sunshine duration of approximately 2 638.8 h.

The soil type is yellow-brown earth, neutral to slightly alkaline, with a relatively thick layer and good fertility. The soil contains available nitrogen at 90 - 120 mg/kg, available phosphorus at 15 - 25 mg/kg, and available potassium at 100 - 150 mg/kg. The organic matter content ranges from 20 to 25 mg/kg, and the soil is relatively rich in mineral elements such as calcium, magnesium,

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and iron. The soil pH is approximately 6.0 – 7.5.

Experimental materials

The tested hawthorn variety was 'Tieshanzha'. The trees were approximately 18 years old They were selected from a hawthorn orchard with an area of 30 hm^2 using a multi-point random sampling method. A total of 36 healthy trees with uniform growth vigor, consistent tree shape, height, trunk thickness, no pests or diseases, and normal fruiting were chosen and individually numbered.

The experimental fertilizer used was Dugao fish protein peptide purchased from Shenzhen Dugao Biotechnology Co., Ltd. Related parameters of the product were as follows: free amino acid content 120 g/L, enzymatic hydrolysate of fish protein 50%, Zn + B ≥20 g/L, EDTA-Zn 10 g/L boron 10 g/L. The product was presented as an aqueous solution.

Experimental design

Three key factors closely related to yield, namely, fertilization timing, application method, and application concentration, were selected. Each factor was tested at three levels using an orthogonal test design, and four replicates were set for each treatment group. The fertilization periods were: June 1 – 10 (early growth stage), July 15 – 25 (core-hardening stage), and September 10 – 15 (pre-coloring stage). The application methods included root application, foliar spray, and root application + foliar application. Fish protein peptide concentrations were set at 0 (control), 5, and 10 ml/L. The solution was diluted to target concentrations, and the total mixture volume for each treatment was 1 L. Each solution was transferred into a spray bottle. For foliar application, the entire plant was sprayed uniformly using an atomizing nozzle, preferably between 4:00 PM and sunset. For root application, a circular trench 30 cm deep and 30 cm wide was dug at a distance of 1 m from the trunk of each hawthorn tree. The fish protein peptide aqueous solution was applied into the trench, followed by covering with soil and compacting.

Design method of orthogonal test

Factor level design An orthogonal test was designed using the orthogonal array $L_9(3^4)$. The factor levels for various treatments are shown in Table 1.

Table 1 Factor and level table

Level	Factor		
	Application time A	Application method B	Application concentration C//ml/L
1	Fruit-setting stage	Root application	0
2	Core-hardening stage	Foliar spray	5
3	Pre-coloring stage	Root application + foliar spray	10

Design of orthogonal test The design of the orthogonal test is shown in Table 2.

Fruit quality measurement methods and data processing

Fruit quality measurement methods Internal quality index of fruit: Vitamin C (Vc) content was determined by the 2,6-dichlorophenol indophenol titration method[7].

External quality index: Single fruit weight was measured using an electronic balance (TP-1102, Denver Instrument Co., Ltd.). In specific, 20 hawthorn berries were collected from southeast, northeast, southwest, and northwest directions, respectively, after ripening. The collected fruit was weighed, and averaged[8]. The results were expressed in grams (g) and accurate to 0.01 g.

Table 2 $L_9(3^4)$ design

Experiment No.	Factor A	B	C	D
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	2	1	2	3
5	2	2	3	1
6	2	3	1	2
7	3	1	3	2
8	3	2	1	3
9	3	3	2	1

Data processing Data were organized using Excel and analyzed with SPSS. The results were subjected to variance analysis and significance testing. When $P < 0.05$, the differences between groups were considered statistically significant and marked with lowercase letters.

Table 3 Analysis of $L_9(3^4)$ orthogonal test results for single fruit weight of hawthorn berries

Test No.	A	B	C	D	Single fruit weight//g
1	1	1	1	1	13.19
2	1	2	2	2	13.83
3	1	3	3	3	13.07
4	2	1	2	3	11.10
5	2	2	3	1	10.21
6	2	3	1	2	10.02
7	3	1	3	2	9.59
8	3	2	1	3	11.02
9	3	3	2	1	11.73
K_1	40.09	33.88	34.23	35.13	
K_2	31.33	35.06	36.66	33.44	
K_3	32.34	34.82	32.87	35.19	
k_1	13.36	11.29	11.41	11.71	
k_2	10.44	11.69	12.22	11.15	
k_3	10.78	11.61	10.96	11.73	
MAX	13.36	11.69	12.22	11.73	
MIN	10.44	11.29	10.96	11.15	
R_j	2.92	0.39	1.26	0.58	
Order of effect	A > C > B				
Optimal combination	$A_1B_2C_2$				

Results and Analysis

Effects of different treatments on external quality (single fruit weight) of hawthorn berries

Orthogonal test results for single fruit weight The orthogonal test results for single fruit weight of hawthorn berries are shown in Table 3. Range analysis revealed the influence hierarchy of the three factors on single fruit weight. Fertilization timing (factor A) had the most significant effect, followed by application concentration (factor C), while application method (factor B) showed the least effect. The order of main effects was: A > C > B. For factor A, level 1 (fruit-setting stage) showed the highest average value at 13.36, significantly exceeding other periods. For factor B, level 2 (foliar spray) demonstrated the optimal average value of 11.69. For factor C, level 2 (5 ml/L) yielded the best result with an average value of 12.22. The optimal combination was determined as $A_1B_2C_2$, indicating foliar spraying at 5 ml/L during the fruit-setting stage as the most effective approach.

The three-factor ANOVA in Table 4 revealed that factor A (application time) had a significant effect on single fruit weight, with its sum of squares accounting for 82% of the total variation, indicating this factor serves as the core regulatory element for fruit weight increase. Factor C (application concentration) did not reach the statistical significance level, while factor B (application method) showed the least effect.

Significance test of differences between various levels of treatment factors The results of significance test for differences between application time levels are shown in Table 5. The results for various fertilization methods are shown in Table 6. Those for application concentrations are shown in Table 7.

Table 4 Analysis table of variance for orthogonal design

Three-factor analysis of variance					
Source of variation	Sum of Squares	Degrees of freedom	Mean square	*F*	Significance
Application time A	15.31	2	7.66	23.27	0.04*
Fertilization method B	0.26	2	0.13	0.39	0.72
Application concentration C	2.46	2	1.23	3.74	0.21
Error	0.66	2	0.33		
Total	18.69				

$R^2 = 0.965$

* $P < 0.05$ $P < 0.01$.

Table 5 *LSD* multiple comparisons on the effects of application time on hawthorn fruit weight

Application time			Mean difference	Standard error	Significance	95% confidence interval	
						Lower limit	Upper limit
LSD	1	2	2.92*	0.47	0.03	0.90	4.94
		3	2.58*	0.47	0.03	0.57	4.60
	2	1	-2.92*	0.47	0.03	-4.94	-0.90
		3	-0.34	0.47	0.55	-2.35	1.68
	3	1	-2.58*	0.47	0.03	-4.60	-0.57
		2	0.34	0.47	0.55	-1.68	2.35

Table 6 *LSD* multiple comparisons on the effects of application methods on hawthorn fruit weight

Application time			Mean difference	Standard error	Significance	95% confidence interval	
						Lower limit	Upper limit
LSD	1	2	-0.39	0.47	0.49	-2.41	1.62
		3	-0.31	0.47	0.57	-2.33	1.70
	2	1	0.39	0.47	0.49	-1.62	2.41
		3	0.08	0.47	0.88	-1.94	2.10
	3	1	0.31	0.47	0.57	-1.70	2.33
		2	-0.08	0.47	0.88	-2.10	1.94

Table 7 *LSD* multiple comparisons on the effects of application concentrations on hawthorn fruit weight

Application concentration			Mean difference	Standard error	Significance	95% confidence interval	
						Lower limit	Upper limit
LSD	1	2	-0.81	0.47	0.23	-2.83	1.21
		3	0.45	0.47	0.44	-1.56	2.47
	2	1	0.81	0.47	0.23	-1.21	2.83
		3	1.26	0.47	0.11	-0.75	3.28
	3	1	-0.45	0.47	0.44	-2.47	1.56
		2	-1.26	0.47	0.11	-3.28	0.75

From Table 5, Table 6 and Table 7, it can be concluded that the differences between various application time (fruit-setting stage, core-hardening stage and pre-coloring stage) reached extremely significant levels, while the difference between the fruit-setting stage and pre-coloring stage was not significant. Among fertilization methods (root application, foliar spray, and root application + foliar spray), no significant differences were observed between any treatments. Similarly, no significant differences were found among various application concentrations (0, 5, and 10 ml/L).

Effects of different treatments on the Vc content in hawthorn fruit

Orthogonal test results of Vc content in hawthorn fruit The orthogonal test results for Vc content in hawthorn fruit are presented in Table 8. Range analysis revealed the order of influencing factors as: B > C > A, *i. e.*, application method > application concentration > application time. Factor B had the largest range (12.37), indicating that fertilization method had the most significant effect on Vc content, followed by application concentration, while application time had a relatively weaker influence. The optimal combination was $A_2B_2C_2$, meaning the highest Vc content (98.91 mg/100 g) was achieved when the fertilizer was applied during the core-hardening stage (A_2) by root application + foliar spray (B_2) at a concentration of 10 ml/L (C_2).

From the perspective of main effect significance, the three-factor ANOVA in Table 9 shows that factor B (fertilization method) had an extremely significant effect on the target trait, with a sum of squares of 280.83, accounting for 66.1% of the total variation from the three factors, making it the dominant factor. Factor C (application concentration) had a significant effect, contributing 30.5% and playing an important regulatory role in the target trait. In contrast, factor A (application time) had no significant effect on the target trait. Through comparative analysis on mean square values, the order of factor effects was determined as B > C > A. Among them, factor B was 2.17 times more influential than factor C, indicating greater potential for improving the target trait through optimized fertilization methods.

Table 8 Analysis of $L_9(3^4)$ orthogonal test results for Vc content in hawthorn fruit

Test No.	A	B	C	D	Average Vc content mg/100 g
1	1	1	1	1	81.85
2	1	2	2	2	88.61
3	1	3	3	3	95.4
4	2	1	2	3	76.47
5	2	2	3	1	98.91
6	2	3	1	2	94.92
7	3	1	3	2	83.76
8	3	2	1	3	91.67
9	3	3	2	1	85.51
K_1	265.86	242.08	268.44	266.27	
K_2	270.30	279.19	250.59	267.29	
K_3	260.94	275.83	278.07	263.54	
k_1	88.62	80.69	89.48	88.76	
k_2	90.10	93.06	83.53	89.10	
k_3	86.98	91.94	92.69	87.85	
MAX	90.10	93.06	92.69	89.10	
MIN	86.98	80.69	83.53	87.85	
R_j	3.12	12.37	9.16	1.25	
Order of effect			B > C > A		
Optimal combination			$A_2B_2C_3$		

Table 9 Analysis of variance for orthogonal design

Three-factor analysis of variance					
Source of variation	Sum of Squares	Degrees of freedom	Mean square	F	Significance
Application time A	14.61	2	7.31	5.83	0.15
Fertilization method B	280.83	2	140.42	112.06	0.01
Application concentration C	129.61	2	64.81	51.72	0.02 *
Error	2.51	2	1.25		
Total	425.06				

R^2 = 0.994

* $P<0.05$; $P<0.01$.

Significance test of differences between treatment factor levels

The LSD multiple comparisons of application time effects on the Vc content in hawthorn fruit are presented in Table 10, showing no significant differences between all treatment levels.

The effects of fertilization methods on the Vc content of hawthorn fruit were analyzed using the *LSD* multiple comparison test (Table 11, with specific index values included). The foliar spray treatment significantly increased Vc content by 12.37 mg/100 g compared with treatment B_1. The root application and foliar spray combined treatment resulted in an Vc content 11.25 mg/100 g higher than the root application alone. These findings indicated that both treatments B_2 and B3 were effective fertilization methods. However, treatment B_2 significantly and substantially enhanced Vc content compared with treatment B_1.

The effects of application concentration on the Vc content of hawthorn fruit were analyzed using the LSD multiple comparison test (Table 12). A concentration of 5 ml/L increased Vc content by 5.95 mg/100 g compared with 0 ml/L, while 10 ml/L further increased it by 9.16 mg/100g compared with 5 ml/L. However, the difference between 10 and 0 ml/L was not statistically significant.

The Vc content increased with the application concentration increasing, and 10 ml/L was the optimal level.

Table 10 ***LSD*** **multiple comparisons on the effects of application time on the Vc content of hawthorn fruit**

Application time			Mean difference	Standard error	Significance	95% confidence interval	
						Lower limit	Upper limit
LSD	1	2	-1.48	0.91	0.25	-5.41	2.45
		3	1.64	0.91	0.21	-2.29	5.57
	2	1	1.48	0.91	0.25	-2.45	5.41
		3	3.12	0.91	0.08	-0.81	7.05
	3	1	-1.64	0.91	0.21	-5.57	2.29
		2	-3.12	0.91	0.08	-7.05	0.81

Table 11 ***LSD*** **multiple comparisons on the effects of application methods on the Vc content of hawthorn fruit**

Application method			Mean difference	Standard error	Significance	95% confidence interval	
						Lower limit	Upper limit
LSD	1	2	-12.37*	0.91	0.01	-16.30	-8.44
		3	-11.25*	0.91	0.01	-15.18	-7.32
	2	1	12.37*	0.91	0.01	8.44	16.30
		3	1.12	0.91	0.35	-2.81	5.05
	3	1	11.25*	0.91	0.01	7.32	15.18
		2	-1.12	0.91	0.35	-5.05	2.81

Table 12 ***LSD*** **multiple comparisons on the effects of application concentrations on the Vc content of hawthorn fruit**

Application concentration			Mean difference	Standard error	Significance	95% confidence interval	
						Lower limit	Upper limit
LSD	1	2	5.95*	0.91	0.02	2.02	9.88
		3	-3.21	0.91	0.07	-7.14	0.72
	2	1	-5.95*	0.91	0.02	-9.88	-2.02
		3	-9.16*	0.91	0.01	-13.09	-5.23
	3	1	3.21	0.91	0.07	-0.72	7.14
		2	9.16*	0.91	0.01	5.23	13.09

Conclusions and Discussion

In this study, the regulatory effects of different fertilization methods and application time on hawthorn physiology and fruit quality were investigated. The results demonstrated that the synergistic treatment of root application and foliar spray (T_5) exhibited significant advantages at a concentration of 5 ml/L, which was closely related to the dual-channel nutrient supply mode. Root application ensured sustained absorption of mineral elements, while foliar spraying rapidly supplied photosynthetic enzyme activators during critical phenological stages. Their synergistic effect promoted the translocation of carbon assimilates to the fruit, which aligns with the findings of Yuan's study on apples[9]. Spraying amino acid foliar fertilizer significantly improved the quality of greenhouse cherry[10], increasing single-fruit weight. Foliar application also enhanced the trees' vegetative growth, elevated leaf mineral nutrient content, improved fruit quality, and effectively inhibited disease occurrence[11].

Based on the above analysis, appropriate application time, methods, and concentrations enhanced hawthorn fruit quality. Among these factors, application time had the greatest impact on single fruit weight, with foliar spraying during the fruit-setting stage at 5 ml/L proving most effective. Fertilization methods and concentrations had relatively minor effects on hawthorn fruit quality. The order of effects on hawthorn fruit's internal quality (Vc content) was fertilization method > application concentration > application time. The optimal combination was $A_2B_2C_3$, *i.e.*, foliar spraying at a concentration of 10 ml/L during the core-hardening stage yielded the best fertilization effect.

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