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Effect of Different Ratios of Cow Manure and Chemical Fertilizers on Fruit Quality of Gala Apples

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Abstract: Nutrient patterns can significantly influence the growth and development of fruit trees, especially fruit quality. In order to clarify the appropriate ratio of inorganic and organic fertilizers, six treatments with different ratios of cow manure and chemical fertilizers were set up in this study to evaluate the effects of different treatments on fruit yield, appearance quality, intrinsic quality and volatile substances. The results showed that, after replacing some chemical fertilizers with cow manure, the content of sugar, titratable acid, organic acid and aroma substance in the fruit increased, the fruit yield and colour did not change significantly, and the fruit weight per fruit decreased. Among the treatments of cow manure with chemical fertilizer, the fruits of 50% chemical fertilizer with 50% cow manure treatment had higher titratable acid (0.4%), malic acid (4.15 mg/g), sorbitol (0.51%), glucose (1.30%), fructose (5.81%) content and total aroma substance content (1047.82 µg/g) than chemical fertilizer alone. Compared to the fertilizer treatment alone, the application of cow manure instead of 50% of the chemical fertilizer had a positive effect on fruit quality.



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Keywords: apple; cow manure; fertilizer; fruit quality

1. Introduction

The apple cultivation area and production of China are the first in the world. According to Food and Agriculture Organization of the United Nations (FAO) [1], the world apple production is basically stable between 83–87 million tons, and the planted area is about 4.6 million hectares in 2020. Meanwhile, the Chinese apple production is basically stable between 39 and 42 million tons, and the planted area is about 1.9 million hectares, accounting for 47% and 41% of the world's apple production and area, respectively in 2020. This shows that China's apple industry occupies an important position in the world apple industry, and it plays a pivotal role in the precise poverty alleviation in remote mountainous areas, rural revitalization and farmers' income enrichment. However, with the development and growth of the Chinese apple industry, the contradiction between large production and difficult sales has become increasingly serious, affecting the enthusiasm of growers and the sustainable and healthy development of the industry. Previous research demonstrated that there were many factors influencing the stagnation of apples; one of the important factors was the lack of structural supply of high-quality apples. Not only was the high-quality fruit one of the connotations of high-quality development in the fruit industry, but consumers were also more concerned about it [2,3]. This was reported in research that besides the variety, many factors such as soil conditions, climate, cultivation management measures and plant protection measures all had a great influence on the quality of apples, among which, the scientific fertilization technology of cultivation management measures played a key role in the formation of apple quality, which can directly affect the fruit size, colour, sugar and acid, aroma and other related quality indicators [4–7].

Some authors had suggested that the application of organic fertilizer increased the weight per fruit as well as the number of fruits of apples [8,9]. A two-year experiment in southern Brazil by Amarante et al. (2008) [10] confirmed that the application of organic fertilizers resulted in a more yellow base colour and a higher percentage of red skin in apple fruit. In addition, Liu et al. (2018) [11] reported soluble solids content increased with the amount of organic fertilizer applied, and although the differences between treatments were not significant, they were all higher than the control group. Meanwhile, the results of Zhao et al. (2013) [12] and Zhang et al. (2020) [13] confirmed that a higher soluble solids content and a lower titratable acid of apple were observed in the reasonable application of organic and inorganic fertilizer in the cultivation of apples that compared to the application of organic or inorganic fertilizers alone.

Fruit flavour quality is relatively complex, and we perceive the flavour of the fruit through our senses of smell, taste, etc. It is an important component of the comprehensive quality of the fruit, which has an important guiding role for consumers to choose fruit. The flavour of the fruit is mainly influenced by the type and content of sugars, acids and aromatic substances in the fruit [14–18]. The organic sugar acids mainly include sucrose, fructose, sorbitol, glucose, malic acid, quinic acid and mangiferic acid, while the fruit aroma substances mainly include esters, alcohols, aldehydes, ketones, ethers, terpenoids and alkanes. Zhao et al. (2019) [19] showed that bio-organic fertilizers applied in combination with chemical fertilizers could increase the content of reducing sugar fraction in jujube fruits. Another study confirmed that the application of organic fertilizer could increase the glucose and sucrose content and decrease the malic, succinic, tartaric and total acid content in the fruits of Feicheng peach [20,21]. Meanwhile, the results of Wang et al.'s study showed that the application of organic fertilizers resulted in a wider variety and higher relative content of aromatic substances compared to apples fertilized with chemical fertilizers [22]. It has been reported that the application of chicken manure and chemical fertilizers together can promote the synthesis of aroma substances in melon fruits [23], but it has also been shown that the combination of organic and inorganic fertilizers did not have a significant effect on compounds affecting fruit aroma compared to the application of chemical and organic fertilizers alone [24].

At present, there are many studies on the effect of organic fertilizers and inorganic fertilizers on fruit quality, but the pattern of fruit flavour quality changes under different fertilization treatments is not very consistent; especially, the study on the flavour quality of apples with cow manure and chemical fertilizers is rarely reported. Therefore, considering the climate changes associated with global warming, understanding the effect of different ratios of cow manure applied in combination with chemical fertilizers on apple fruit flavour may enable selecting appropriate orchard management practices to achieve high fruit quality.

2. Materials and Methods

2.1. Site Description

The current long-term fertilization site was established in 2017 by Chinese Academy of Agricultural Sciences, situated in Xingcheng, Liaoning province, China (120.73° E, 40.70° N). The climate of the site is mild-temperate, semi-humid, with total precipitation of 620 mm and mean annual temperature of 11 °C in 2020 [25]. The soil type of the orchard was brown soil with sandy texture. General physio-chemical properties of the soil before the beginning of the experiment were: bulk density 1.51 g/cm³, pH 6.05, electrical conductivity 0.08, SOC 10.37 g/kg, total N 0.51 g/kg, and available P, and K, 3.65 and 8.14 mg/kg. 'Gala'/SH38/*Malus baccata* (L.) Borkh, planted in 2017 with plants spaced 4 × 1.5 m, were used in this study. The entire experimental field was 0.15 hm² and each cultivation density of fruit trees was 1250 trees/hm².

2.2. Experimental Design and Field Management

The long-term fertilization experiment was conducted using a randomized block design, having three replications. Six different treatments were implemented in the present study: (1) control check (CK), (2) 100% chemical fertilizer alone (100% CF), (3) 75% chemical

fertilizer with 25% cow manure (75% CF + 25% CM), (4) 50% chemical fertilizer with 50% cow manure (50% CF + 50% CM), (5) 25% chemical fertilizer with 75% cow manure (25% CF + 75% CM), and (6) 100% cow manure alone (100% CM). Every five trees were divided into one block, with three trees in the middle as test trees and two trees at the edge as protection trees. Each block was replicated three times and randomly arranged within the block. Fertilizers included well-rotted cow manure and chemical fertilizer (urea(N-P-K is 46-0-0), potassium sulfate(0-0-50), diammonium phosphate(18-48-0)). N content of cow manure was 1.41% in 2017, 1.83% in 2018, 2.79% in 2019, 1.62% in 2020. According to Wang et al. (2016) [26], the nitrogen dosage was 240 kg/hm² at the young tree stage (2017–2018) and 480 kg/hm² in 2019–2020.

The cow manure was calculated based on its N content, and the specific weight of application was calculated. The cow manure was applied at once in autumn. Chemical fertilizer is applied in three periods each year, with one-third of the nitrogen, one-quarter of the phosphorus and one-fifth of the potassium applied in the spring during the slow growing period, one-third of the nitrogen, one-quarter of the phosphorus and three-fifths of the potassium applied during the fruit expansion period, and one-third of the nitrogen, one-half of the phosphorus and one-fifth of the potassium applied in the autumn after the fruit has been harvested. The fertilizer is applied in furrows, in the middle of the canopy projection. The specific amounts (pure nutrients) applied in 2017–2020 are shown in Table 1.

Table 1. Nitrogen, phosphorus, potassium and organic fertilizer inputs for different treatments (kg/hm²).

Treatment	2017–2018					2019–2020				
	N	P ₂ O ₅	K ₂ O	Cow Manure (N)	Total N	N	P ₂ O ₅	K ₂ O	Cow Manure (N)	Total N
CK	0	0	0	0	0	0	0	0	0	0
100% CF	240	120	240	0	240	480	240	480	0	480
75% CF + 25% CM	180	90	180	60	240	360	180	360	120	480
50% CF + 50% CM	120	60	120	120	240	240	120	240	240	480
25% CF + 75% CM	60	30	60	180	240	120	60	120	360	480
100% CM	0	0	0	240	240	0	0	0	480	480

The sampling of the fruits took place in the second half of August 2020. For each treatment, 45 apples from three trees (15 fruits per tree) were hand-harvested (from the middle part and the entire perimeter area of each tree) at physiological maturity, and immediately transported to the laboratory. Once in the laboratory, a selection of the 30 most homogeneous fruits (size, shape, and colour) was made and 10 of these were separated for the weight, size, colour, total soluble solids (TSS), total titratable acidity (TA) and the other 20 fruits were used for organic acids, sugars, and volatile compounds determinations.

2.3. Physical Chemical Analysis, Organic Acids, and Sugar Fraction

A total of 10 apple fruits from each treatment were selected to determine colour, size, total soluble solids (TSS), total titratable acidity (TA), and weight. TSS were determined by digital refractometer (PLA-1, Japan); total titratable acids and vitamin C (VC) were determined by 905 automatic potentiometric titrator. The fruit surface colour of samples was measured using a precision handheld colourimeter (NR20XE, China). Fruit surface colour mainly consisted of five indicators: the L* value represents the brightness of the colour, and the value range is from 0 to 100, whereby 100 represents white and 0 represents black; a* represents the red and green degree, and the value range is from −60 to +60; a* is red and purple when positive, and green and blue when negative, and the larger the absolute value is, the darker the colour is; b* represents the yellow and blue degree, and the value range is also from −60 to +60; so, 42 is yellow when positive, and blue when negative, and the larger the absolute value is, the darker the colour is. From the a* and b* values, C* (Chroma) and H⁰ (hue angle) can be calculated. Where $C^* = [a^{*2} + b^{*2}]^{1/2}$ indicates the colour saturation and H⁰ is the hue angle. When a* > 0 and b* > 0, $H^0 = \tan^{-1}(b^*/a^*)$; when a* < 0 and b* > 0, $H^0 = 180 + \tan^{-1}(b^*/a^*)$; when h⁰ = 0 indicates red, H⁰ = 90° indicates yellow, and H⁰ = 180° indicates green [27].

Organic acids and sugars were identified and quantified according to Sánchez-Bravo et al. [28] with some modifications. The apple juices were prepared by hand-squeezing in a commercial juicer. Then, the juices were centrifuged at $12,000 \times g$ for 30 min (HITACHI RXII, Japan), filtered through a $0.45 \mu\text{m}$ Millipore filter, and injected (1.5 mL) into a high-performance liquid chromatograph (HPLC) system. Organic acids were separated on a Supelcogel C-610H column ($30 \text{ cm} \times 7.8 \text{ mm}$) fitted with a Supelcoguard column ($2.5 \text{ cm} \times 4 \text{ mm}$) and a diode array detector (210 nm) was used for the detection. For the determination of sugars, the same HPLC system and conditions were used, but detection was performed with a refractive index detector. Standard curves of pure organic acids and sugars were used for quantification. The concentrations of organic acids and sugars are expressed in mg/g and %, respectively.

2.4. Aroma Substances

The fruit aroma substances and their contents were determined by headspace extraction combined with gas chromatography-mass spectrometry.

Headspace extraction combined with gas chromatography-mass spectrometry (GC-MS) was performed on an Agilent 7890A/5975C gas chromatograph-mass spectrometer.

Sample pretreatment: The fruit samples were taken, cleaned, cut and de-seeded, diced, and sampled by the quadratic method. A total of 200 g of samples were crushed and homogenized with a juicer, 10 g of homogenate was weighed in a 20 mL headspace vial, 1 g of sodium chloride and 5 μL of 0.4 g/L 3-nonanone were added, sealed in a glander, and stored at $-80 \text{ }^\circ\text{C}$ for measurement.

Headspace extraction: The sample vial was equilibrated at a constant temperature of $40 \text{ }^\circ\text{C}$ in a water bath for 10 min, and the Supelco 50/30 μm PDMS/CAR/DVB solid phase microextraction head was inserted into the headspace portion of the sample vial and extracted at $40 \text{ }^\circ\text{C}$ in a water bath for 30 min.

GC-MS measurement conditions: the column was HP-5MS ($30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$); the carrier gas was high-purity helium with a flow rate of 1.3 mL/min. manual injection, no splitting mode. Inlet temperature was $250 \text{ }^\circ\text{C}$. Column temperature ramp-up procedure: initial $40 \text{ }^\circ\text{C}$, hold for 3 min, ramp up to $140 \text{ }^\circ\text{C}$ at $3 \text{ }^\circ\text{C}/\text{min}$; ramp up to $240 \text{ }^\circ\text{C}$ at $10 \text{ }^\circ\text{C}/\text{min}$, hold for 3 min, mass spectrometry interface temperature at $250 \text{ }^\circ\text{C}$, ion source temperature at $230 \text{ }^\circ\text{C}$, full scan mode, and scan range: 40~550 amu.

The data were analyzed using the chromatographic workstation that comes with the system, and the integration parameters were: initial area truncation 10,000, initial peak width 0.1, shoulder peak detection OFF, and initial threshold 5.0. The spectrum library was selected from the NIST 11 standard library. The internal standard method was used for the quantitative analysis of the aroma components, and the content of the substances was expressed as the amount of three-nonanone.

Calculation formula ($\mu\text{g}/\text{g}$ FW) = sample peak area $\times 0.4 \times 5 \times 10^{-6}$ / 3-nonanone peak area / sample mass $\times 10^9$ (Sample mass is generally 10 g).

2.5. Statistical Analysis

Data statistics using Excel from Microsoft 365. A one-way analysis of variance (ANOVA) was performed for the analysis of the results. STATISTICAL ANALYSIS SYSTEM 9.4 (SAS9.4) was used for the statistical treatment of the data, LSD for post hoc test and the significant difference was defined as $p < 0.05$.

3. Results and Discussion

3.1. Single Fruit Weight, Yield, Longitudinal Diameter, Diameter and Colour

This experiment showed that the single fruit weight of each fertilization treatment was significantly higher than that of the CK treatment without fertilization, and Table 2 shows the effect of different ratios of cow manure and chemical fertilizers on fruit weight per fruit and longitudinal and transverse diameter.

Table 2. Effect of different ratios of cow manure and chemical fertilizers on single fruit weight, yield, longitudinal diameter and transverse diameter.

Treatment	Single Fruit Weight (g)	Yield (kg/acres)	Longitudinal Diameter (mm)	Transverse Diameter (mm)
100% CF	173.18 ± 2.85 a	393.80 ± 73.39 a	61.68 ± 0.55 a	70.37 ± 0.71 a
75% CF + 25% CM	170.82 ± 6.24 ab	348.44 ± 41.64 ab	60.83 ± 0.38 ab	70.40 ± 0.94 a
50% CF + 50% CM	171.92 ± 6.79 ab	340.65 ± 37.16 ab	60.32 ± 0.09 b	68.85 ± 0.76 ab
25% CF + 75% CM	166.26 ± 3.83 ab	377.24 ± 61.89 a	60.14 ± 0.16 b	68.27 ± 0.81 b
100% CM	163.24 ± 4.83 b	418.54 ± 35.52 a	60.12 ± 1.16 b	67.99 ± 0.51 b
CK	127.21 ± 6.77 c	265.21 ± 35.47 b	56.53 ± 0.67 c	64.51 ± 1.21 c
ANOVA	*	*	*	*

Note: Values followed by different letters, within the same column, were significantly different (* means that $p < 0.05$).

For fruit weight per fruit, it was lower in all treatments with cow manure than with chemical fertilizer alone, with the 50% CF + 50% CM treatment having the highest fruit weight per fruit (171.92 g) among the treatments. Fruit weight per fruit was significantly higher in the chemical fertilizer treatment than in the cow manure treatment, but there was no significant difference between the other fertilizer treatments. The trend in fruit longitudinal diameter and diameter were similar to that of fruit weight. Fruit yield was higher in all fertilizer treatments than in the CK treatments, with no significant differences between fertilizer treatments.

Colour, size, and shape are often factors contributing to consumers' buying decisions [29]. The results of this study showed that the application of cow manure alone, chemical fertilizer and cow manure and chemical fertilizer combination were able to increase fruit weight per fruit compared to the no fertilizer control. Fruit weight per fruit was significantly lower with cow manure alone than with chemical fertilizer alone; whereas, there was no significant difference between the treatments with cow manure and chemical fertilizer in combination with chemical fertilizer and cow manure alone. Fertilizer application could increase fruit weight per fruit, but different fertilizers supplied nutrients in slightly different ways. Chemical fertilizers have faster nutrient release efficiency while the nutrient types are more fixed; whereas, cow manure has slower nutrient release but its nutrient composition is richer. Another study by Khorram et al. (2018) [30] also concluded that there was no significant change in fruit weight per fruit with biochar, cow manure compost and biochar + cow manure compost fertilizers.

We find that the fruit colour indexes (Tables 3 and 4) differed little between treatments. The study showed that peel colour is the preferred trait to determine the market acceptability of a variety, and the fruit colour of apple is mainly determined by its base colour, followed by anthocyanin pigmentation. Factors such as light, temperature, mineral nutrition, growth regulators, and carbohydrate utilization all affect anthocyanin accumulation. Light intensity and low temperature play a crucial role in anthocyanin accumulation in apple pericarp [31].

Table 3. Effect of different ratios of cow manure and chemical fertilizers on the colour of the sunny side of fruits.

Treatment	L *	a *	b *	C *	H ⁰
100% CF	53.99 ± 5.86	36.23 ± 2.50	15.91 ± 2.55	39.41 ± 1.96	22.82 ± 4.73
75% CF + 25% CM	51.44 ± 2.06	39.96 ± 0.96	13.88 ± 0.36	42.39 ± 0.89	19.39 ± 0.82
50% CF + 50% CM	49.60 ± 1.25	40.55 ± 1.23	14.40 ± 1.16	43.33 ± 0.41	19.66 ± 1.98
25% CF + 75% CM	53.34 ± 2.92	37.52 ± 1.71	13.55 ± 1.56	40.11 ± 3.73	19.69 ± 3.60
100% CM	49.74 ± 5.19	40.19 ± 1.24	14.94 ± 1.57	43.03 ± 2.38	20.24 ± 2.85
CK	54.64 ± 1.51	36.63 ± 2.86	15.82 ± 2.63	40.33 ± 1.36	23.30 ± 5.54
ANOVA	NS	NS	NS	NS	NS

Note: Values followed by different letters, within the same column, were significantly different (* means that $p < 0.05$). NS means no significant difference.

Table 4. Effect of different ratios of cow manure and chemical fertilizers on the colour of the shade side of fruits.

Treatment	L *	a *	b *	C *	H ⁰
100% CF	67.79 ± 4.23	22.41 ± 2.41	22.08 ± 2.67	32.09 ± 0.99	45.21 ± 4.98 a
75% CF + 25% CM	70.47 ± 0.38	21.81 ± 1.06	21.59 ± 0.91	31.06 ± 0.76	46.29 ± 5.06 a
50% CF + 50% CM	64.03 ± 0.20	28.86 ± 5.93	19.75 ± 2.33	34.52 ± 1.83	30.06 ± 3.02 bc
25% CF + 75% CM	68.57 ± 3.12	23.33 ± 6.39	19.69 ± 2.63	31.13 ± 2.76	36.02 ± 3.86 b
100% CM	62.94 ± 8.78	29.36 ± 9.72	19.51 ± 4.03	35.77 ± 3.91	26.38 ± 2.93 c
CK	70.22 ± 1.22	20.80 ± 2.47	23.42 ± 2.82	32.33 ± 1.73	50.27 ± 4.57 a
ANOVA	NS	NS	NS	NS	*

Note: Values followed by different letters, within the same column, were significantly different (* means that $p < 0.05$). NS means no significant difference.

3.2. Total Soluble Solids, Titratable Acid, Sugar-Acid Fraction

Fruit flavour quality are significantly influenced by sugar and acid content, so metabolic regulation of sugar and acid plays a key role in fruit development. Figure 1 shows the effect of different ratios of cow manure and fertilizer on fruit glycolic acid and its components.

Total soluble solids (Figure 1A) and titratable acid (Figure 1B) contents of fruits were not significantly different among fertilizer treatments, but were higher than those of non-fertilizer treatments. Previous studies have shown that organic fertilizer treatments can increase the titratable acid content of fruits [32]. In the present study, the titratable acid content of fruits was higher in all fertilizer treatments than in the non-fertilizer treatments, and the titratable acid content of fruits in different ratios of organic fertilizer and chemical fertilizer were higher in all treatments than in chemical fertilizer alone, which is consistent with the results of previous studies on lemon that organic lemons had a lower titratable acidity concentration than lemons grown in a conventional way [33]. It has also been suggested that organic fertilizer can reduce fruit acid content [34], that organic fertilizer application can reduce titratable acid content in passion fruit [35], and that organic fertilizer replaces chemical fertilizer to reduce titratable acid content in apple fruit [36].

There are various fractions of sugars (Figure 1C–F) in apple fruit, mainly four, including sorbitol, glucose, fructose, and sucrose, with the highest percentage of fructose and sucrose. There are three main organic acid fractions (Figure 1G–I) in the fruit, including malic acid, quinic acid, shikimic acid, of which malic acid has the highest percentage and the rest of the acids are low [37]. Malic acid is an apple-specific acid and therefore the main source of sourness in apples, and quinic acid affects the astringency of apple fruit and plays an important role in the perception of sweetness [38]. A total of four sugars and three organic acids were identified in the apples of this study, in agreement with previous studies [39]. Fructose and sucrose accounted for a higher proportion of the sugar fraction, and malic acid was the highest in the organic acid fraction. Among the fertilization treatments, 50% CF + 50% CM treatment had higher content of each sugar fraction in the fruits.

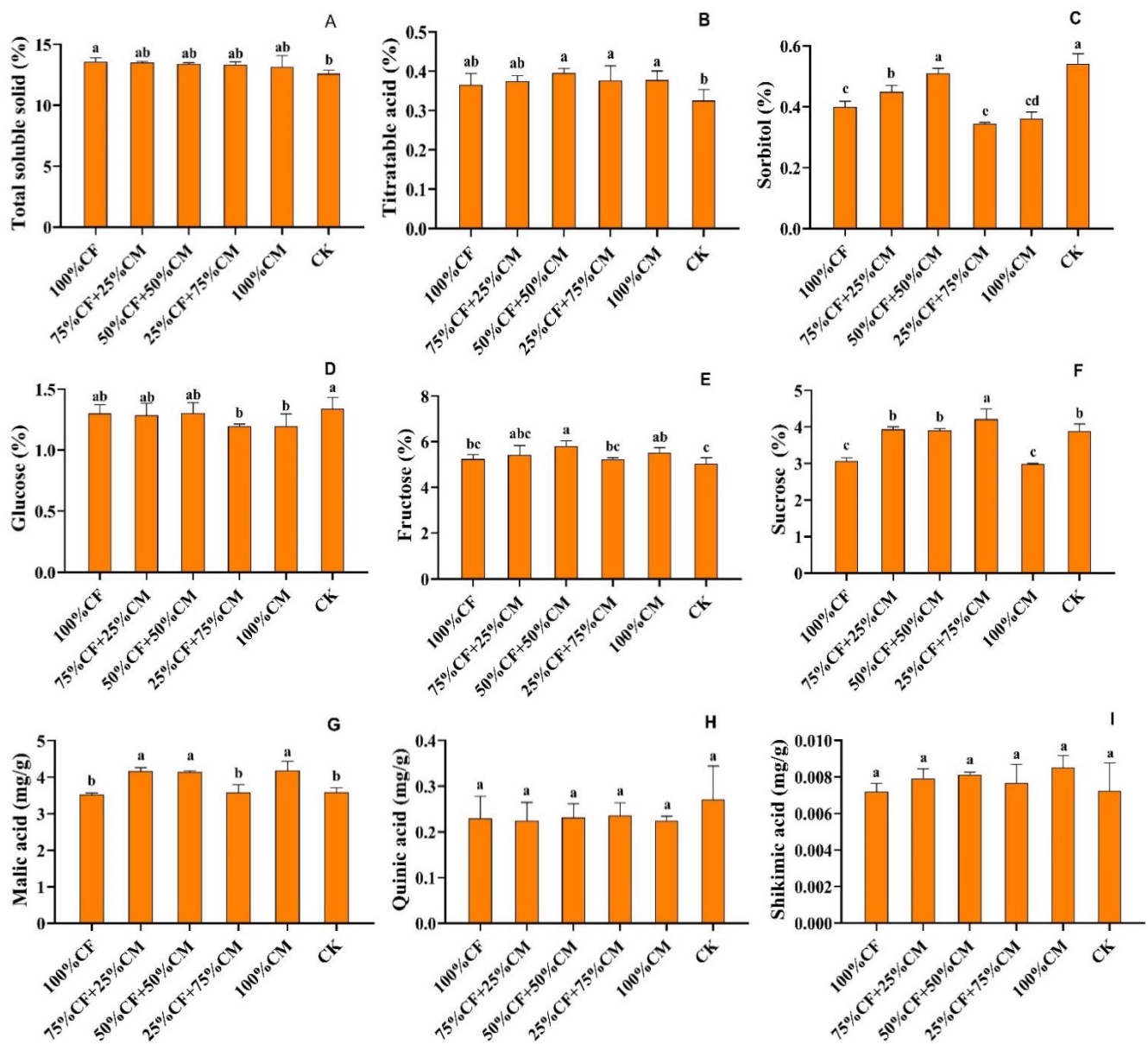


Figure 1. Effect of different ratios of cow manure and chemical fertilizers on total soluble solids, titratable acid, sugar-acid fraction of fruits: (A) total soluble solid; (B) titratable acid (C) sorbitol; (D) glucose; (E) fructose; (F) sucrose; (G) malic acid; (H) quinic acid; (I) shikimic acid. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

The results of this study showed that the treatment of cow manure with chemical fertilizer could increase the fructose and sucrose contents of fruits, and significantly increase the malic acid content of fruits compared with chemical fertilizer alone. The results were consistent with previous studies on blueberry fruits [40].

3.3. Aroma Substances

A total number of 26 volatile compounds were identified in all the studied apples, as shown in Table 5, with the highest proportion of ester aroma substances, followed by alcohol aroma substances, as well as a small amount of aldehyde aroma substances, and a very small amount of ether and ketone aroma substances. The three most abundant esters were butyl acetate, hexyl acetate and 2-methylbutyl acetate.

Table 5. Effect of different ratios of cow manure and chemical fertilizers on fruit aroma substances.

Aroma Substances	100% CF	75% CF + 25% CM	50% CF + 50% CM	25% CF + 75% CM	100% CM	CK	ANOVA
Butyl acetate (µg/g)	199.11 ± 23.15 b	277.14 ± 19.23 a	274.84 ± 9.94 a	269.53 ± 12.60 a	252.56 ± 16.61 a	178.54 ± 6.19 b	*
Hexyl acetate (µg/g)	196.30 ± 13.43 d	224.17 ± 11.61 c	287.16 ± 12.19 a	257.84 ± 15.22 b	241.23 ± 17.70 bc	147.51 ± 17.14 e	*
2-Methylbutyl acetate (µg/g)	57.08 ± 8.45 c	101.08 ± 0.63 a	100.51 ± 7.10 a	105.41 ± 6.31 a	83.57 ± 7.60 b	41.58 ± 6.78 d	*
Butyl hexanoate (µg/g)	33.67 ± 0.99 b	29.99 ± 1.72 c	39.00 ± 1.30 a	34.69 ± 0.68 b	33.96 ± 1.08 b	18.16 ± 0.04 d	*
Pentyl acetate (µg/g)	22.31 ± 1.81 d	27.56 ± 0.60 bc	31.46 ± 0.01 a	29.12 ± 0.14 b	27.07 ± 1.59 c	18.22 ± 0.72 e	*
Hexanoic acid hexyl ester (µg/g)	22.25 ± 0.40 b	10.99 ± 0.02 c	27.14 ± 3.72 a	20.53 ± 2.27 b	20.46 ± 3.28 b	11.33 ± 1.46 c	*
Butyl butyryl lactate (µg/g)	15.66 ± 7.65	16.12 ± 2.50	23.79 ± 8.23	21.09 ± 2.60	18.52 ± 12.39	16.53 ± 4.68	NS
Hexyl-2-methylbutyrate (µg/g)	15.78 ± 1.61 b	14.89 ± 0.00 b	24.25 ± 1.81 a	21.45 ± 2.20 a	17.38 ± 2.85 b	9.69 ± 0.85 c	*
Butyl propionate (µg/g)	3.69 ± 0.56 ab	3.75 ± 0.49 ab	4.65 ± 0.15 a	3.73 ± 0.98 ab	3.37 ± 0.62 b	1.37 ± 0.25 c	*
Ethyl-2-methylbutyrate (µg/g)	2.69 ± 1.80	2.63 ± 0.79	3.30 ± 1.36	3.77 ± 0.99	2.35 ± 2.51	1.48 ± 0.39	NS
Ethyl acetate (µg/g)	0.81 ± 0.16 c	1.52 ± 0.41 ab	1.93 ± 0.27 a	1.38 ± 0.35 abc	0.90 ± 0.01 c	1.10 ± 0.23 bc	*
Methyl hexanoate (µg/g)	0.96 ± 0.70	1.05 ± 0.46	1.56 ± 0.83	1.12 ± 0.68	0.80 ± 0.51	0.82 ± 0.23	NS
Octyl butyrate (µg/g)	0.63 ± 0.29	0.68 ± 0.16	0.98 ± 0.39	0.86 ± 0.11	0.75 ± 0.43	0.60 ± 0.11	NS
Hexanol (µg/g)	97.80 ± 6.03 b	77.22 ± 0.66 c	113.58 ± 4.46 a	108.86 ± 1.60 a	66.13 ± 3.29 d	71.63 ± 2.21 cd	*
1-Butanol (µg/g)	35.61 ± 2.89 a	34.28 ± 2.75 a	38.37 ± 3.73 a	28.04 ± 4.16 b	27.39 ± 4.01 b	26.93 ± 0.11 b	*
2-Methyl alcohol (µg/g)	10.22 ± 3.06 a	8.89 ± 1.43 a	2.46 ± 0.96 b	9.09 ± 0.48 a	8.67 ± 0.12 a	3.22 ± 0.54 b	*
5-Hexen-1-ol (µg/g)	7.73 ± 3.29	9.02 ± 2.10	9.86 ± 1.75	9.08 ± 1.36	7.61 ± 6.06	7.45 ± 2.03	NS
3-Nonanol (µg/g)	1.86 ± 0.08	1.85 ± 0.05	1.81 ± 0.20	1.64 ± 0.11	1.34 ± 0.82	1.62 ± 0.21	NS
3-Hexen-1-ol (µg/g)	1.25 ± 0.16 ab	1.20 ± 0.50 ab	1.60 ± 0.12 a	1.41 ± 0.31 a	0.90 ± 0.11 b	1.17 ± 0.34 ab	*
1-Octanol (µg/g)	0.65 ± 0.42	0.73 ± 0.47	0.88 ± 0.31	0.69 ± 0.25	0.78 ± 0.64	0.54 ± 0.15	NS
Phenethyl alcohol (µg/g)	0.22 ± 0.13	0.31 ± 0.11	0.33 ± 0.15	0.26 ± 0.08	0.29 ± 0.10	0.25 ± 0.12	NS
2-Hexanal (µg/g)	19.58 ± 1.39 e	32.38 ± 1.98 b	34.33 ± 1.92 a	27.34 ± 0.26 c	26.66 ± 1.36 c	23.14 ± 1.46 d	*
Hexanal (µg/g)	8.41 ± 1.11 bc	9.90 ± 0.06 b	12.19 ± 0.41 a	8.37 ± 1.25 bc	3.66 ± 0.67 d	7.83 ± 1.53 c	*
2-Heptenal (µg/g)	1.35 ± 0.76 b	1.82 ± 0.91 ab	3.55 ± 1.57 a	1.88 ± 1.26 ab	0.68 ± 0.15 b	2.20 ± 1.07 ab	*
Benzaldehyde (µg/g)	0.25 ± 0.09 b	0.49 ± 0.38 ab	0.60 ± 0.19 a	0.46 ± 0.16 ab	0.34 ± 0.15 ab	0.46 ± 0.20 ab	*
4-Allyl anisole (µg/g)	3.90 ± 0.16 a	3.16 ± 0.79 ab	3.69 ± 0.51 ab	1.56 ± 0.14 c	3.05 ± 0.38 b	1.47 ± 0.22 c	*
6-Methyl-5-hepten-2-one (µg/g)	1.33 ± 0.58 bc	1.73 ± 0.44 bc	4.01 ± 1.74 a	1.91 ± 0.91 bc	1.06 ± 0.65 c	3.02 ± 1.43 ab	*
Total aroma substance (µg/g)	761.07 ± 7.44 d	894.57 ± 36.45 c	1047.82 ± 31.08 a	971.09 ± 33.91 b	851.49 ± 43.03 c	597.84 ± 29.14 e	*

Note: Values followed by different letters, within the same row, were significantly different (* means that $p < 0.05$). NS means no significant difference.

The fruit of 100% CM treatment contained 252.56 µg/g of butyl acetate, which was significantly higher than that of 100% CF treatment. There was no significant difference in the content of butyl acetate in the cattle manure with chemical fertilizer treatment, but all of them were significantly higher than that of the chemical fertilizer treatment alone. The 241.23 µg/g of hexyl acetate in the 100% CM treatment was significantly higher than that of the 100% CF treatment. Among the cow manure with fertilizer treatments, the fruit hexyl acetate content of 50% CF + 50% CM treatment was 287.36 µg/g, which was significantly higher than the other treatments. Fruit 2-methylbutyl acetate content was not significantly different among the treatments. The content of most of the remaining aroma substances was also higher in the 50% CF + 50% CM treatment. Most of the fruit aroma substances were higher in the treatments of cow manure with chemical fertilizer than in the treatments of chemical fertilizer alone and cow manure alone.

The substances affecting apple aroma were mainly composed of various volatile organic compounds, including terpenes, aldehydes, alcohols, esters, ketones and sulfur-containing compounds. However, not all volatile substances contribute to fruit aroma, but only when their substance content reaches a certain value will they have an impact on fruit aroma. The variety of aromatic substances in plants is extremely rich, and more than 2000 species have been identified. The type and content of aroma substances vary among different plant species. There are at least four pathways from primary metabolites to the formation of various aromatic compounds [41]. Fatty acid and amino acid pathways are the two main metabolic pathways for the synthesis of esters in fruit. The production of esters aromatic volatiles is controlled by ethylene [42]. Esters are the most abundant of the volatile substances in apples. Nitrogen supply plays a role in the formation of aroma, which is limited under nitrogen deficient conditions. Fatty acids and amino acids are important substrates for the synthesis of aroma substances [43]. Therefore, we speculate that the input of nitrogen fertilizer can promote the synthesis of precursors such as amino acids in the fruit, which in turn affects the synthesis of aroma substances.

Previous studies have shown that different fertilization treatments significantly affect the content of aroma substances in fruits [24], and studies on dates found higher levels of sugars, acids, and aroma substances under organic treatments [44]. In our study, it was found that esters accounted for an extremely high percentage of apple fruit aroma substances, about 75%, followed by alcohols, about 20%, and the rest were aldehydes, ethers, and ketones. Among the esters, butyl acetate, hexyl acetate, and 2-methylbutyl acetate were higher. The 50% CF + 50% CM treatment had the highest content of these three species and total esters, and the ester content of organic fertilizer with chemical fertilizer treatment was significantly higher than that of chemical fertilizer alone and CK treatment, indicating that the application of organic fertilizer could significantly increase the accumulation of esters in fruits. The content of total aroma substances in fruits was also higher in the 50% CF + 50% CM treatment, which was significantly higher than that of the chemical fertilizer alone and the CK treatment. Therefore, the synthesis of various aroma substances in fruits was promoted by the treatments of cow manure and chemical fertilizer, with the 50% CF + 50% CM treatment having the most significant enhancement effect.

4. Conclusions

Compared to chemical fertilizer application alone, treatments with 50% CF + 50% CM showed higher sugar, titratable acids, organic acids, and aroma substance content. However, it should be noted that treatment with organic fertilizer alone had a negative impact on apple single weight compared to 100% CF treatment. No significant effect of different treatments on fruit colour was shown. Overall, the five-year fertilization experiment showed that the application of cow manure instead of 50% of the chemical fertilizer had a positive effect on fruit quality compared to 100% CF treatment.

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