https://doi.org/10.17221/69/2024-HORTSCI

Effect of leaf-to-fruit ratio on kernel quality formation of walnut trees

Linhui Feng, Xian'an Yang, Shiwei Wang, Honglong Hao, Cuifang Zhang, Yicheng Gao, Yuqi Wang, Yusufu Alifu, Wenhui Zhao*

Department of Forestry, College of Forestry and Landscape Architecture, Xinjiang Agricultural University, Urumqi, Xinjiang, P.R. China

**Corresponding author: zcf851022@163.com*

Citation: Feng L.H., Yang X.A., Wang S.W., Hao H.L., Zhang C.F., Gao Y.C., Wang Y.Q., Alifu Y., Zhao W.H. (2025): Effect of leaf-to-fruit ratio on kernel quality formation of walnut trees. Hort. Sci. (Prague), 52: 201–210.

Abstract: The study focused on the relationship between leaf-to-fruit ratio (LFR) and walnut kernel quality in *Juglans regia* 'Wen 185'. It was investigated how LFR influences single kernel weight, the contents and percentages of organic matter in kernels, the ^{13}C distribution proportion ($^{13}C_{DP}$) in crude fat and protein, and the number and size of oil bodies within the kernels. A gradually decreasing LFR led to reduced single kernel weight, the contents of crude fat, crude protein, and soluble sugar dramatically ($P < 0.05$), with no significant changes in the percentages and ($^{13}C_{DP}$) of crude fat, crude protein, and soluble sugar ($P > 0.05$). Moreover, there were no significant differences in the number of oil bodies per unit area and the size of oil bodies in kernel cotyledons and endosperm storage cells among the different LFR ($P > 0.05$). We propose that the walnut kernel quality depends on the proportion of sugar converted into fat and protein in the kernels, that the changes in LFR affect the amount of sugar accumulated in kernels but not the proportion of sugar converted to fat and protein, and that the LFR, therefore, have no effect on the percentages of crude fat and crude protein in walnut kernels.

Keywords: embryo; oil accumulation; stable isotope; submicroscopic structure

The theory of source-sink relationships is often used in modern economic plant cultivation physiology research to clarify the law of economic plant yield and quality formation. The yield of economically important plants depends on the production capacity of photosynthetic source assimilates and the size and capacity of the sink (Slfer et al. 2023). Changes in the source-sink relationship would affect plants' physiological and metabolic activities, including the function and development of leaves and the transportation and distribution of assimilates (Zhang et al. 2024). The coordinated source-sink relationship significantly improves economic plant yield (Wang et al. 2020a). A higher biomass yield and an optimised source-sink relationship will result in the next great leap in economic plant yields (Qin et al. 2023). The change in the source-sink relationship affects the redistribution of assimilates within economic plants, not only on economic plant yield but also on their quality. For economically important plants where the goal is to harvest seeds, the formation of organic matter (e.g. starch, fat, and protein) in seed kernels requires photosynthesis to provide raw materials or energy. The number of photosyn-

Supported by the Natural Science Foundation of Xinjiang Uygur Autonomous Region of China, No. 2022D01A74.

https://doi.org/10.17221/69/2024-HORTSCI

thetic sources (leaves) and their photosynthetic rates affect organic matter synthesis. However, the regulatory effect of source-sink relationship changes on the content of organic matter in kernels is different (Rivelli et al. 2024), which may be related to plant species, organic matter species, source-sink regulation range, and growth and development periods.

Most studies indicate that the protein content in plant kernels is most affected by changes in the source-sink relationship. It was shown that the grain protein content in maize was the most sensitive to the source reduction, followed by the starch content, using different varieties (Wang et al. 2020b). The changes in the source-sink relationship significantly affected the grain protein content of spring wheat but had little effect on starch and soluble sugar content. Meanwhile, the correlation between protein content and the source-sink relationship differed among the different varieties. Starch accumulation is mainly limited by the sink, and protein content is limited by both the source and sink (Liu 2014). The protein content in grains of indica-japonica hybrid rice at maturity was higher than in conventional japonica rice, which may be due to an imbalance in the source-sink relationship, resulting in more N in leaves being transferred to grains (Wei et al. 2018).

However, the effects of the source-sink relationship on fat content in plant kernels are different. Some studies have suggested that the source-sink relationship does not affect the fat content in plant kernels. For example, the fat content in maize grains of different varieties is relatively stable under different source-sink relationships (Wang et al. 2020b), the relationship between the fat percentage in maize grains and the source-sink ratio is not close and is less affected by source-sink regulation (Shekoofa et al. 2013). Similar results have been reported in sunflowers (Andrianasolo et al. 2016), *Camellia oleifera* (Zhu et al. 2015), and olives (Yuan et al. 2015). However, some studies have suggested that the source-sink relationship affects fat content in plant kernels. For example, total defoliation at the filling stage of soybean is conducive to protein accumulation but not fat accumulation; 1/3 of soybean pods were removed at the seed-filling stage, and the fat accumulation increased, while the protein content decreased (Zhang et al. 2022). By appropriately reducing the source, peanut varieties with large sources could promote the conversion of sugars into fat in kernels and improve the crude fat content in kernels and peanut varieties with multiple sinks, thereby increasing the crude fat content in kernels (Gao 2021).

Through a comprehensive analysis of the above literature, it was found that researchers usually use the leaf-to-fruit ratio (LFR) to represent source-sink relationships and utilise content (g) or percentage (%) as evaluation indicators to determine the impact of source-sink regulation on kernel quality. If the evaluation indices are different, the results will differ. Walnut is an important woody oil tree species, and its kernels are rich in fat and protein. Does the quality of walnut kernels change after a change in the source-sink relationship? How does it change? Therefore, the present study puts forward the hypotheses that the quality of walnut kernels depends on the proportion of sugar converted into fat and protein in the kernels, that the changes in LFR only affect the amount of sugar accumulation in kernels but not the proportion of sugar conversion to fat and protein, and that the LFR, therefore, have no effect on the percentages of crude fat and crude protein in walnut kernels.

MATERIAL AND METHODS

Experimental site and plant materials

The fruits of *Juglans regia* 'Wen 185' were selected in this experiment. The walnut sprouted at the end of March, the leaves appeared in early April, the flowering period was from mid-April to early May, and the fruit matured in late August. The walnut is an early-bearing variety with strong stress resistance. The material used in this experiment is a grafted tree, and the rootstock is raw walnut. The fruits were collected from the walnut production park of Awati county in Aksu region of Xinjiang (78°23'12"–80°01'30"E; 0°43'46"–41°51'05"N) from April 2022 to September 2022 and from April 2023 to September 2023. The average altitude of this area is approximately 1 200–2 000 m, and it is dry and rainless. The annual average temperature, precipitation and evaporation are 10.4 °C, 91.5 mm, and 2 003.8 mm, respectively. The annual sunshine duration is 2 679–2 750 h, and the frost-free period is 211–250 days. The soil is mostly sandy loam. The trees were grown at a spacing of 5 m × 6 m in north-south rows and were all 10 years old.

The experimental site is in the irrigation oasis of the Aksu area, and the water quantity is sufficient. When the maximum field water capacity is less than

https://doi.org/10.17221/69/2024-HORTSCI

60%, irrigation is needed. The plant material needs to be irrigated five times during the growth process. The first irrigation was in late March to promote the germination of leaf buds and flower buds; the second was in late May and early June to promote the growth of fruits; the third was in early July to promote the formation of kernel oil; the fourth was in early August to promote the formation of kernel oil; the fifth was in early October before the soil froze. The effect is to improve the cold resistance of the tree. The average annual irrigation is 1 270 mm.

Collection and processing of walnut kernel organic matter accumulation samples

Samples were collected at fixed points at regular intervals starting 10 days after the end of female flower pollination (DAF) (April 26th), and 30 normally developing fruits were randomly picked every 10 days. The kernels were removed, dried in an electric constant temperature blast drying oven (DHG-9140A, Shanghai Haixiang instrument and equipment factory, China) to kill green at 105 °C for 30 min, and dried at 70 °C to constant weight. After cooling, the kernel dry weight was measured using an electronic balance [ME203E, Mettler Toledo instruments (Shanghai) Co., Ltd., China] to obtain the average value. Finally, the kernel was crushed and passed through a 100-mesh nylon sieve to determine crude fat, crude protein, and soluble sugar content.

Crude fat extraction. Samples (2–5 g) were mixed with sea sand, if necessary, and transferred into a filter paper cylinder. The filter paper cartridge was placed into the fat extractor (JC-SSTQ2, Qingdao Jingcheng Instrument Co., Ltd., China), connected the dried to constant weight receiving bottle, add anhydrous ether or petroleum ether to 2/3 of the volume of the bottle from the upper end of the extraction condensing pipe of the extractor, and heated in a water bath to continuously reflux the ether or petroleum ether for extraction for 6–12 hours. The receiving bottle was removed, recovered using ether or petroleum ether, and evaporated in a water bath until 1–2 mL of ether remained in the receiving bottle. It was then dried at 95–105 °C for 2 hours. Thereafter, it was placed into a dryer (Jiangsu Runhong Science and Education Equipment Co., Ltd., China) and cooled for 0.5 h before being weighed.

Soluble sugar extraction. Ground dry sample (0.05–0.10 g) and 2 mL of water were added to a 5 mL covered plastic centrifuge tube, extracted in a water bath at 80 °C for 10 min, and centrifuged (5430R, Eppendorf SE, Germany) at 3 000 rpm for 5 minutes. The supernatant was carefully transferred to a 10 mL graduated tube with a stopper and extracted twice with distilled water (2 mL at a time). After centrifugation, the supernatant was collected, combined, and diluted to 10 mL with distilled water for ^{13}C determination of soluble sugars using liquid chromatography isotope ratio mass spectrometry (LC-C-IRMS) (Flash 2000 HT O/H-NC and Finnigan Delta V Advantage, Thermo Fisher Scientific, Inc., USA).

Protein extraction. Kernel powder (2 g) was added to deionised water at a solid-liquid ratio of 1 : 11, stirred, and put into an ultrasonic oscillator (KQ-500DE, Kunshan Ultrasonic Instrument Co., Ltd., China) with a power of 500 W and a temperature of 45 °C for 45 minutes. The pH was adjusted to 9 with 1 mol/L NaOH, and the samples were centrifuged (5430R, Eppendorf SE, Germany) at 4 000 rpm for 20 minutes. The supernatant was removed, and the pH was adjusted to approximately 5 with 1 mol/L HCl. The sample was centrifuged at 5 000 rpm for 15 minutes. The precipitates were removed, washed and neutralised with deionised water, and dried to a constant weight in a 60 °C oven (DHG-9140A, Shanghai Haixiang Instrument and Equipment Factory, China).

LFR regulation, sample collection and processing

Defoliation, girdling, and defruiting were performed on sun-exposed shoots with fully expanded leaves and developing fruits on the southern sides of 15 trees after fruit set. Fifteen fruit-bearing shoots per tree were modelled to represent one of the following five LFR value treatments (1L : 1F, 2L : 1F, 3L : 1F, 4L : 1F, and 5L : 1F). The specific operation for the attainment of these LFR treatments, representing sink-source experimental manipulation, was performed according to our previous study (Zhang et al. 2018).

After ripening, all the fruits from the different LFR treatments were picked. The kernels were dried to determine the kernel dry weight, and the crude fat, crude protein, and soluble sugar in the kernels were extracted to calculate the contents (g) and percentages (%) of crude fat, crude protein, and soluble sugar. Three trees in each LFR were used in duplicate, and the average value of 15 fruits per tree was calculated.

Feeding walnut leaves with $^{13}CO_2$ during the critical period of oil accumulation

After fruit setting, three 10-year-old walnut trees were selected for LFR regulation and $^{13}CO_2$ supplementation. Each tree was set up with five LFR (1L : 1F, 2L : 1F, 3L : 1F, 4L : 1F, and 5L : 1F), and

https://doi.org/10.17221/69/2024-HORTSCI

each LFR treatment was applied to four bearing shoots. Based on the information presented earlier, the key period for walnut seed oil accumulation was determined. During the critical period of oil accumulation, the whole walnut tree was fed $^{13}CO_2$ in sunny and windless weather from 11 a.m. to 1 p.m. (Beijing local time). Three trees were fed for each treatment for three consecutive days (July 16th, 17th, and 18th).

$^{13}CO_2$ feeding method

During feeding, $Na_2{}^{13}CO_3$ (with a ^{13}C abundance of 98%) was used as the marker at a dose of 8 g. The entire canopy was sealed in a 2 m × 2 m × 2 m polyethene plastic room with good light transmittance (sunlight transmittance was 95% of the natural light intensity), and a fan (DP-Y17, Shenzhen Liangpin Electronic Technology Co., Ltd., China) and marker were placed in the room. At the beginning of marking, the fan was turned on, and 10 mL of dilute sulfuric acid (1 mol/L) was injected into a beaker every 0.5 h to maintain the $^{13}CO_2$ concentration. During ^{13}C marking, the plastic room was sealed for 3 hours. An appropriate number of ice bags were distributed around the room to control the temperature between 28 °C and 37 °C. A sufficient NaOH (0.4 mol/L) solution containing phenol-phthalein was added to the feeding room after feeding to collect all $^{13}CO_2$ not absorbed by the sample plants.

Sample collection and determination index

Adipiscing destructive sampling was conducted on the morning of July 21st (72 h after the end of the third feeding period). All fruits with the same LFR on the same tree were collected and washed with deionised water, and their kernels were removed and divided into two parts. The first part was dried and weighed according to the method described earlier, and then passed through a 0.25 sieve. The crude fat, crude protein, and soluble sugar of kernels with different LFR were extracted to determine the $\delta^{13}C$. The unfed walnut trees were selected as the control to obtain naturalness $\delta^{13}C$. The calculation formula is as follows:

$$\delta^{13}C = (R_{sample}/R_{PDB} - 1) \times 1\,000$$

where: $\delta^{13}C$ – the abundance of ^{13}C in the sample (‰); R_{sample} – the ratio of ^{13}C and ^{12}C isotopic abundances of the sample; R_{PDB} – the PDB (Peedee belemnite) standard, that is, the ratio of ^{13}C to ^{12}C isotopic abundances in the Cretaceous Peedee Formation of South Carolina, USA, is 0.0112372.

Determination and calculation of ^{13}C contents of crude fat, crude protein, and soluble sugar (^{13}C amount):

$$^{13}C_{amount} = AT\%^{13}C \times C\% \times 0.1$$

where: $^{13}C_{amount}$ – the content of ^{13}C in the sample (mg/g); $AT\%^{13}C$ – the percentage of ^{13}C atoms in the sample (%); $C\%$ – the mass fraction of C atom in the sample (%); 0.1 – 0.1 g sample.

Transmission electron microscope observation

The second part of the kernel sample was used for transmission electron microscope (H-600A, Hitachi, Ltd., Japan) observation. The embryos and cotyledons of kernels with different LFR were trimmed into 5 mm × 2 mm × 2 mm small pieces and quickly immersed in 2.5% glutaraldehyde fixed solution, then washed three times with 0.1 mol/L phosphoric acid buffer with pH = 7.2, fixed for 4 h with 1% osmium tetroxide, and washed three times with 0.1 mol/L phosphoric acid buffer with pH = 7.2, then dehydrated with ethanol followed by acetone transition, Spurr penetration and embedding, and cut into 2–3 μm semi-thin slices and 0.05–0.07 μm ultra-thin slices using an ultra-thin slicer. The diameter and number of oil bodies, and the ratio of oil bodies to the cell cross-sectional area, were observed under a transmission electron microscope.

Data analysis

One-way ANOVA was used to analyse fruit quality, the diameter and quantity of oil bodies, and the ratio of oil bodies to cell cross-sectional area with different LFR, and the least significant difference (LSD) method was used for multiple comparisons. SPSS statistical software version 18.0 was used for data analysis, and Excel software was used to create the graphs.

RESULTS AND ANALYSIS

Accumulation law of organic matter in walnut kernels

The change of protein percentage in the kernel showed a "W" type trend. There were two processes: decline and rise. The crude protein percentage was the highest (23.21%) at 10 DAF and then decreased rapidly at 40 DAF, reaching a peak at 90 DAF. The percentage of crude protein decreased slightly and then changed steadily over the next 40 days, reaching 16.73% at 140 DAF.

https://doi.org/10.17221/69/2024-HORTSCI

The change in crude fat percentage in the kernels showed an increasing trend of "S" type. The crude fat percentage was low and showed little change before 50 DAF. It increased slowly from 50 to 70 DAF and then entered the stage of rapid increase at an average rate of 1.16% from 70 to 110 DAF. The rate of increase slowed from 110 to 140 DAF, and the crude fat percentage reached a maximum value of 65.57% at 140 DAF.

The total soluble sugar percentage in kernels showed an overall trend of first increasing and then decreasing. The overall trend increased and fluctuated from 10 DAF to 60 DAF, reaching the maximum value of 10.93% on 60 DAF. It then fluctuated rapidly from 60 DAF to 110 DAF, and the total soluble sugar percentage decreased by 8.73% at this stage and reached 2.28% at 140 DAF.

The protein content showed a double peak change, the fat content continued to increase, while the total soluble sugar content first increased and then decreased. The period from 70 to 100 days after the final flowering stage represents a critical phase for crude fat synthesis in walnut kernels. Combined with the results of this study and previous studies, it can be concluded that oil and protein anabolism are independent of each other. The sugars and proteins in the kernel will be continuously transformed into crude fat during the growth and development of the walnut fruit (Figure 1).

Analysis of organic matter in walnut kernels with different LFR

There were significant differences among single-kernel weight, crude fat content, crude protein content, and soluble sugar content in walnut kernels from different LFR treatments ($P < 0.05$). These indices increased significantly with increasing LFR ($P < 0.05$). Single-kernel weight (5.81 g), crude fat content (2.88 g), crude protein content (0.91 g), and soluble sugar content (0.35 g) with 5L:1F were significantly higher than those of the other LFR ($P < 0.05$). Single-kernel weight (3.74 g), crude fat content (1.88 g), crude protein content (0.60 g), and soluble sugar content (0.22 g) with 1L:1F were significantly lower than those of other LFR ($P < 0.05$) (Figure 2).

There were no significant differences in crude fat percentage, crude protein percentage, or soluble sugar percentage among kernels with different LFR ($P > 0.05$). The crude fat percentage in kernels was 49.50–53.2%, the crude protein percentage was 14.41–15.98%, and the soluble sugar percentage was 5.85–6.03% (Figure 3).

Content (g) and percentage (%) are different evaluation indices, and the influence results are different. The contents (g) of crude fat, crude protein and soluble sugar in walnut kernel under high LFR treatment were significantly higher, but there was no significant difference in percentage (%).

Proportion of ^{13}C in organic matter in walnut kernels with different LFR

During the critical period of oil accumulation, there was no significant difference in the proportion of ^{13}C converted into crude fat in walnut kernels with different LFR ($P < 0.05$), ranging from 66.42% to 73.20%. There was no significant difference in the proportion of ^{13}C converted into crude protein in walnut kernels with different LFR ($P < 0.05$), ranging from 8.90% to 10.09% (Figure 4).

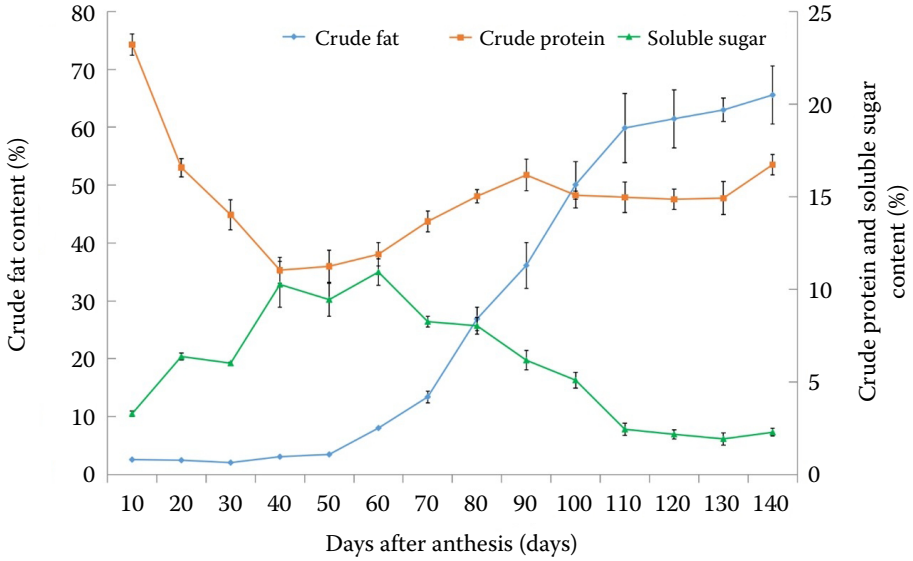

Figure 1. Seasonal variation of organic matter content in walnut kernels

https://doi.org/10.17221/69/2024-HORTSCI

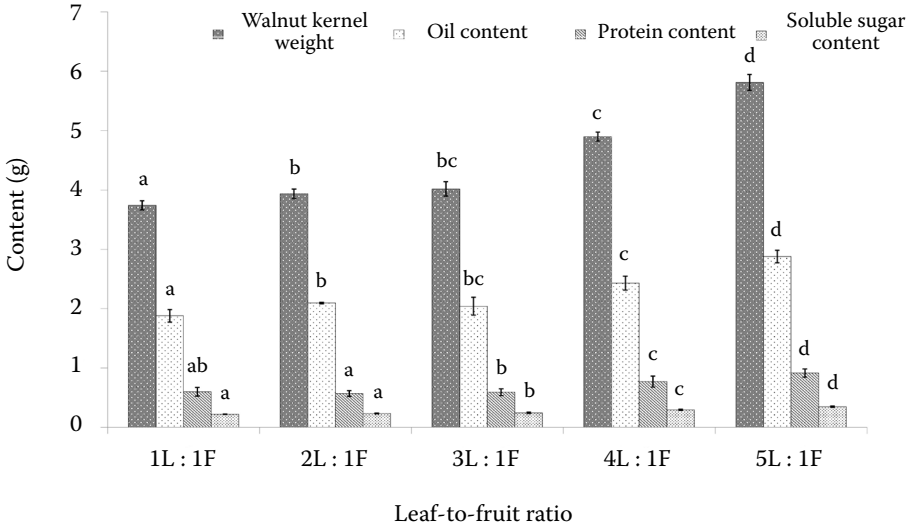

Figure 2. Organic matter content of walnut kernels with different leaf-to-fruit ratios

[a–d]different lowercase letters indicate significant differences at 0.05 level

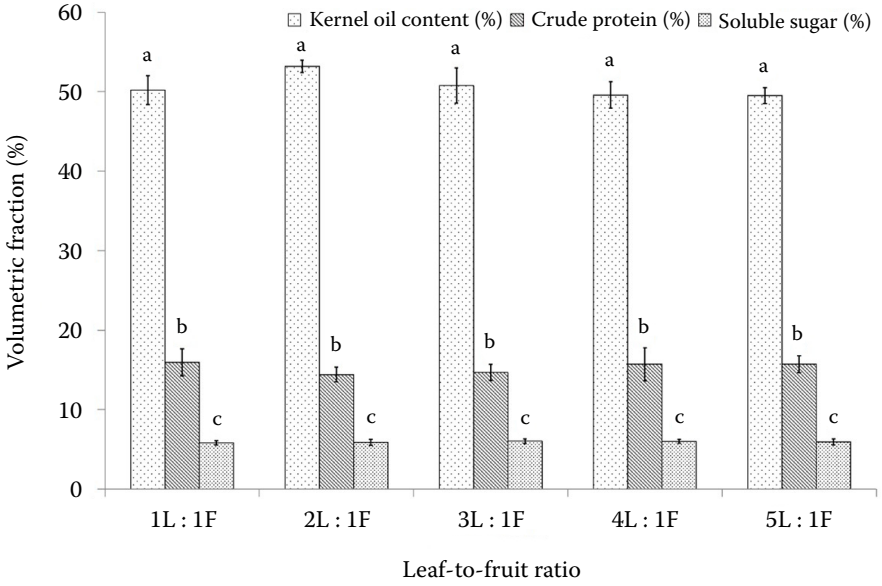

Figure 3. Organic matter content of walnut kernels with different leaf-to-fruit ratios

[a,b,c]different lowercase letters indicate significant differences at 0.05 level

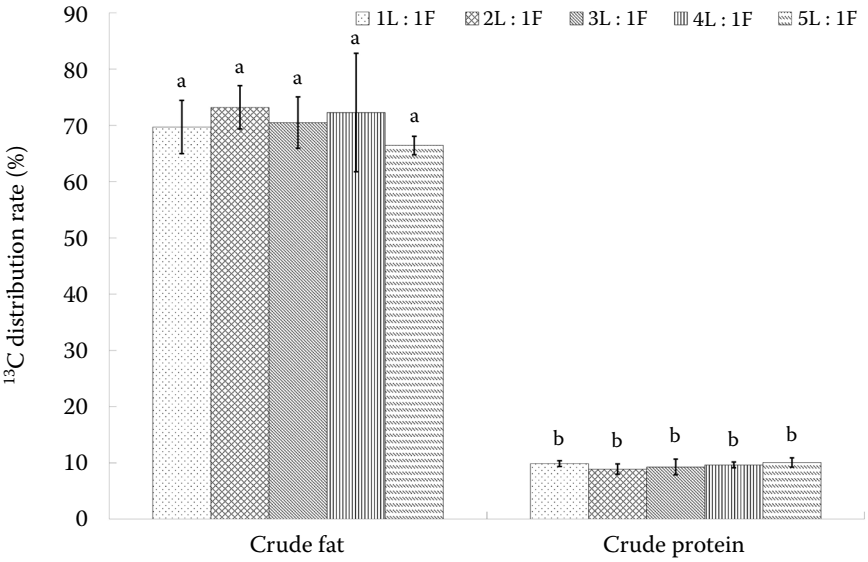

Figure 4. Proportion of ^{13}C in organic matter in walnut kernels with different leaf-to-fruit ratios

[a,b]different lowercase letters indicate significant differences at 0.05 level

https://doi.org/10.17221/69/2024-HORTSCI

Microstructure observation of walnut kernels with different LFR

The crude fat in the kernel is usually stored in the oil bodies of subcellular organelles. Oil bodies can be fixed with osmium tetroxide and stained with heavy metal uranium salt. Light and electron microscopy revealed that the oil body was grey and opaque. During the critical period of oil synthesis, we found that 85 DAF, a large number of oil bodies were observed in the storage cells of walnut embryos with different LFR. However, the sizes of oil bodies were slightly different. Some oil bodies were scattered in the centre of the cells, while others were scattered along the cell wall. Many oil bodies were observed in cotyledon storage cells with different LFR. The number and volume of oil bodies were small, and the oil bodies were independent.

There was no significant difference in the average number, diameter, or average cross-sectional area ratio of the oil bodies in walnut embryo storage cells with different LFR ($P > 0.05$) (Table 1). The average number of oil bodies per 100 μm^2 area ranged from 8.27 ± 0.41 to 8.42 ± 0.18, and the average diameter ranged from 1.29 ± 0.03 to 1.36 ± 0.02 μm. The average sum of the cross-sectional area of the oil body ranged from 49.72 ± 0.22 to 51.01 ± 0.16 μm^2. There were no significant differences in the average number, average diameter, or average cross-sectional area ratio of the oil bodies in walnut cotyledon storage cells with different LFR ($P > 0.05$). The average number of oil bodies per 100 μm^2 area ranged from 6.09 ± 0.17 to 6.22 ± 0.07, and the average oil body diameter ranged from 1.84 ± 0.01 to 1.98 ± 0.02 μm. The average sum of the cross-sectional area of the oil body ranged from 62.98 ± 0.27 to 65.03 ± 0.11 μm^2.

DISCUSSION

The present study showed that the percentage of soluble sugars in walnut kernels increased sharply from 10 to 60 DAF, while the crude fat percentage was low and changed little. The total soluble sugar percentage gradually decreased, and the crude fat percentage gradually increased from 60 to 150 DAF, which is similar to previous research results (Zhang et al. 2001). Studies have shown that sugar in plant kernels participates in the accumulation of crude fat and protein during seed development, and the accumulation of sugar precedes the accumulation of crude fat and gradually decreases with a continuous increase in crude fat accumulation; that is, sugar substances provide raw materials for the synthesis of crude fat (Kambhampat et al. 2021). The results of the present study (Figure 1) show that the critical period of oil accumulation in walnut kernels is from 70 to 100 DAF. Studies have shown that there is a positive correlation between oil content and protein content in walnut kernel, that is, oil anabolism and protein anabolism are independent (Chen et al. 2016), which is similar to the research results in *Xanthoceras sorbifolia* (Zhao et al. 2015) and *Arabidopsis thaliana* (Jasinski et al. 2018). Previous studies have also shown that the accumulation of crude fat in walnut kernels is a process in which sugar and protein in the kernel are continuously transformed into crude fat during the growth and development of walnut fruit; that is, there is "substrate competition" between crude fat and protein (Xie et al. 2008), which is similar to the research results in *Camellia oleifera* (Jiang et al. 2012) and *Brassica napus* (Kennedy et al. 2011). The results of our

Table 1. Comparison of oil body differences of walnut kernels with different leaf-to-fruit ratios

Leaf-to-fruit ratio	Position	Average quantities (pieces)	Average diameter (μm)	The average rate of oil areas and cell areas (%)
1L : 1F	embryos	8.33 ± 0.30^a	1.36 ± 0.02^b	49.72 ± 0.22^b
	cotyledon	6.16 ± 0.12^b	1.98 ± 0.02^a	63.69 ± 0.22^a
2L : 1F	embryos	8.42 ± 0.18^a	1.33 ± 0.01^b	50.03 ± 0.14^b
	cotyledon	6.22 ± 0.07^b	1.84 ± 0.01^a	65.03 ± 0.11^a
3L : 1F	embryos	8.27 ± 0.41^a	1.29 ± 0.03^b	48.62 ± 0.29^b
	cotyledon	6.09 ± 0.17^b	1.94 ± 0.01^a	62.98 ± 0.27^a
4L : 1F	embryos	8.38 ± 0.26^a	1.31 ± 0.03^b	51.01 ± 0.16^b
	cotyledon	6.13 ± 0.17^b	1.89 ± 0.02^a	63.87 ± 0.31^a
5L : 1F	embryos	8.41 ± 0.27^a	1.28 ± 0.03^b	50.13 ± 0.26^b
	cotyledon	6.19 ± 0.11^b	1.92 ± 0.02^a	64.28 ± 0.24^a

^{a,b}different lowercase letters indicate significant differences at 0.05 level

https://doi.org/10.17221/69/2024-HORTSCI

study showed that there were no regular differences between the crude protein and crude fat percentages in walnut kernels during the growth and development of walnut fruit (Figure 1); therefore, we speculated that there was no relationship between fat and protein anabolism in walnut kernels.

The present study found that the content of crude fat, protein, and soluble sugar in walnut kernels with a high LFR was significantly higher ($P < 0.05$) (Figure 2). The single walnut kernel with a high LFR possessed higher quality and larger volume; therefore, the crude fat and protein accumulated in the kernel were higher. We also analysed the percentage of organic matter in kernels with different LFR. A percentage represents a ratio, and the ratio did not change when both the mass of walnut kernels (as denominators) and the mass of crude fat or protein increased; therefore, there was no significant difference among the percentage of crude fat, crude protein, and soluble sugar in walnut kernels with different LFR ($P > 0.05$). These findings are similar to those previously reported for corn (Shekoofa et al. 2013), sunflower (Andrianasolo et al. 2016), and *Camellia oleifera* (Zhu et al. 2015). We speculate that the quality of walnut kernels depends on the proportion of sugar converted into fat and protein. The changes in LFR only affect the amount of sugar accumulation in kernels but do not affect the proportion of sugar conversion to fat and protein. Therefore, LFR did not affect the percentages of crude fat or crude protein in walnut kernels.

We used ^{13}C pulse labelling technology at the critical period of oil accumulation in walnut kernels when feeding walnut trees with different LFR to verify our hypothesis. We found no significant difference in the proportion of ^{13}C converted into the crude fat and protein in walnut kernels with different LFR ($P < 0.05$), indicating that during the critical period of oil synthesis, the photosynthetic products with different LFR were converted into fat and protein in a fixed proportion in the kernel. In the present study, 66.42%–73.20% of the photosynthates were transformed into fat and 8.90%–10.09% into protein at 80 DAF. However, this conversion proportion may change at different stages of oil formation, which is verified later.

The relationships among the crude fat percentage of plant kernels, oil body size, and oil body protein have received considerable attention. Studies have shown that high-oil plant varieties not only contain more oil bodies and a larger sum of cross-sectional areas (Dong et al. 2009) but also have larger oil body diameters than low-oil plant varieties (Yin et al. 2013). The ratio of the oil body to the cell cross-sectional area in walnut kernel storage cells was the main factor affecting the oil percentage difference between high- and low-oil varieties. Paddick and Sprague (1939) attributed the reason why the oil content (%) of corn is not related to the source-sink relationship to the constant embryo/endosperm ratio in maize kernels (Paddick and Sprague 1939). The present study found no significant differences in the average number, diameter, and average cross-sectional area ratio among oil bodies in walnut and cotyledon storage cells with different LFR ($P > 0.05$) (Table 1), which could also be used as evidence that there was no significant difference in the oil content of walnut kernels with different LFR.

CONCLUSION

The sugar in walnut kernels participated in the accumulation of crude fat and protein during seed development, and the accumulation of sugar preceded the accumulation of crude fat and gradually decreased with the continuous increase in crude fat accumulation; that is, sugar-provided raw materials for crude fat synthesis. The critical period of crude fat synthesis in walnut kernels ranges from 70 to 100 DAF; however, there is no relationship between crude fat and protein metabolism in walnut kernels. Single walnut kernels with a high LFR possessed higher quality and larger volume; therefore, the quality of crude fat, crude protein, and crude fat accumulated in the kernel was higher. However, there was no significant difference in the proportion of ^{13}C converted into crude fat or protein in walnut kernels with different LFR ($P > 0.05$). There was no significant difference in the ^{13}C distribution rate of crude fat and crude protein in walnut kernels under different LFR ($P < 0.05$), indicating the critical period of oil synthesis; the photosynthetic products with different LFR were converted into fat and protein in a fixed proportion in the kernel. There was no significant difference in the average number, diameter, or average cross-sectional area ratio of oil bodies in walnut and cotyledon storage cells with different LFR ($P > 0.05$) (Table 1), which could also be used as evidence that there was no significant difference in the oil content of walnut kernels with different LFR.

https://doi.org/10.17221/69/2024-HORTSCI

In this study, content (g) and percentage (%) were used to evaluate the quality of seed kernel with different LFR. It was found that the higher the LFR, the greater the weight of a single kernel, and the higher the content of crude fat and crude protein (g) in the kernel, but the percentage (%) of crude fat and crude protein was not affected by the LFR. ^{13}C isotope pulse labelling technology proved that the distribution ratio of sugar to fat and protein in the kernel was consistent among different LFR treatments at the key period of seed oil formation. Therefore, the change of LFR only affected the amount of sugar accumulation in the kernel, but did not affect the distribution ratio of sugar to fat and protein in the kernel. LFR had no effect on the content of crude fat and crude protein in walnut seeds.

Acknowledgement: Authors thank everyone who contributed their time and effort to the manuscript.

REFERENCES

Andrianasolo F.N., Champolivier L., Debaeke P., Maury P. (2016): Source and sink indicators for determining nitrogen, plant density and genotype effects on oil and protein contents in sunflower achenes. Field Crops Research, 192: 33–41.

Chen H., Pan C., Wang B., Hu Y., Xiao Z., He M. (2016): The relationship among nutrients' accumulation and dynamic changes of fatty acids in seed development of walnut. Journal of Agricultural University of Hebei, 39: 57–62. (in Chinese)

Dong J., Shi D., Gao J., Li C., Liu J., Qi C., Yang W. (2009): Correlation between the quantity and the sum of areas of oil bodies and oil content in rapeseed (*Brassica napus*). Chinese Bulletin of Botany, 44: 79–85. (in Chinese)

Gao F. (2021): Mechanism of yield and quality formation and regulation in different source-sink types of peanut. [Ph.D. Thesis.] Shandong, Shandong Agricultural University: 1–85. (in Chinese)

Jasinski S., Chardon F., Nesi N., Lécureuil A., Guerche P. (2018): Improving seed oil and protein content in *Brassicaceae*: Some new genetic insights from *Arabidopsis thaliana*. Oilseeds and Fats Crops and Lipids, 25: D603.

Jiang Z., Tan X., Yuan J., Ye S., Li Z. (2012): Content variation of main nutrients in leaves and fruits of *Camellia oleifera*. Journal of Central South University of Forestry and Technology, 32: 42–45. (in Chinese)

Kambhampat S., Aznar-Moreno J.A., Bailey S.R., Arp J.J., Chu K.L., Bilyeu K.D., Durrett T.P., Allen D.K. (2021): Temporal changes in metabolism late in seed development affect biomass composition. Plant Physiology, 186: 874–890.

Kennedy Y., Yokoi S., Sato T., Daimon H., Nishida I., Takahata Y. (2011): Genetic variation of storage compounds and seed weight in rapeseed (*Brassica napus* L.) germplasms. Breeding Science, 61: 311–315.

Liu W. (2014): Effects of source sink regulation on carbon and nitrogen metabolism physiology and grain quality of two spike type high yield wheat. [Ph.D. Thesis.] Zhengzhou, Henan Agricultural University: 1–134. (in Chinese)

Paddick M.E., Sprague H.B. (1939): Maize seed characters in relation to hybrid vigour. Journal of the American Society of Agronomy, 31: 743–750.

Qin A., Aluko O.O., Liu Z., Yang J., Hu M., Guan L., Sun X. (2023): Improved cotton yield: Can we achieve this goal by regulating the coordination of source and sink? Frontiers in Plant Science, 14: 1136636. (in Chinese)

Rivelli G.M., Calderini D.F., Abeledo L.G., Miralles D.J., Rondanini D.P. (2024): Yield and quality traits of wheat and rapeseed in response to source-sink ratio and heat stress in post-flowering. European Journal of Agronomy, 152: 127028.

Shekoofa A., Emam Y., Pessarakli M. (2013): Source-sink manipulation effects on maize kernel quality. Journal of Plant Nutrition, 36: 1401–1411.

Slfer G.A., Foulkes M.J., Reynolds M.P., Murchie E.H., Carmo-Silva E., Flavell R., Gwyn J., Sawkins M., Griffiths S. (2023): A 'wiring diagram' for sink strength traits impacting wheat yield potential. Journal of Experimental Botany, 74: 40–71.

Wang Y., Pang Y., Chen K., Zhai L., Shen C., Wang S., Xu J. (2020a): Genetic bases of source-, sink-, and yield-related traits revealed by genome-wide association study in *Xian* rice. The Crop Journal, 8: 119–131.

Wang X., Zheng X., Cao Y., Yu H., Wu T. (2020b): Effects of source alteration on material production and grain nutrition components accumulation of transplanted maize. Journal of Maize Sciences, 28: 111–116. (in Chinese)

Wei H., Meng T., Li X., Dai Q., Zhang H., Yin X. (2018): Sink-source relationship during rice grain filling is associated with grain nitrogen concentration. Field Crops Research, 215: 23–38.

Xie H., Huang Y., Xue X., Xu C., Liu L. (2008): Growth and development of the *Carya cathayensis* nut. Journal of Zhejiang Forestry College, 25: 527–531. (in Chinese)

Yin D., Song J., Zhang X., Li H., Wang X., Cui D. (2013): Microstructure of peanut seeds at different developmental stages. Journal of Nuclear Agricultural Sciences, 27: 344–349. (in Chinese)

Yuan J., Shi B., Wu Z., Tan X. (2015): Response of fruit quality and leaf photosynthesis to different sink-source rela-

https://doi.org/10.17221/69/2024-HORTSCI

tionships in *Camellia oleifera*. Plant Physiology Journal, 51: 1287–1292. (in Chinese)

Zhang C., Pan C., Chen H. (2018): Effects of leaf-to-fruit ratio on chlorophyll fluorescence parameters of walnut (*Juglans regia* L.) leaves. Photosynthetica, 56: 1429–1436. (in Chinese)

Zhang Y., Li W., Han Z., Zhang K., Liu J., Henke M. (2024): Three-dimensional dynamic growth and yield simulation of daylily plants. Smart Agriculture, 6: 140–153. (in Chinese)

Zhang Y., Wang M., Zhao L., Zhang D., Jia Y., Wang J., Li K., Chen L. (2022): Comparison of nutrient composition changes of different soybean varieties during germination. Chinese Journal of Oil Crop Sciences, 44: 1368–1374. (in Chinese)

Zhang Z., Gao Y., Wang W., Zhang Y., Fang Z. (2001): Changes of main nutrients during the fruit ripening of walnut. Acta Horticulturae Sinica, 28: 509–511. (in Chinese)

Zhao N., Zhang Y., Wang J., Liu X., Zhao C., Guo H. (2015): Seed development, lipid accumulation and its relationship with carbohydrates and protein in *Xanthoceras sorbifolia* Bunge. Bulletin of Botanical Research, 35: 133–140. (in Chinese)

Zhu Z., Jiang C., Shi Y., Wu W., Chen N. (2015): Response of yield and leaf photosynthesis to sink-source ratio altering demand in olive. Scientia Agricultura Sinica, 48: 546–554. (in Chinese)

Received: April 10, 2024
Accepted: March 26, 2025
Published online: August 18, 2025