A Quantitative Assay for Fatty Acid Composition of Castor Seed in Different Developmental Stages

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Abstract The aim of this study was to detect and investigate the fatty acid composition of castor seed under different development stages using soxhlet extraction and capillary gas chromatography methods to find the dynamic change of fatty acid composition and their relationships. The results showed that oil content and twelve fatty acid compositions were identified in the seed development, while the content of oil and compositions were significantly different and monitored during seed development. Oil content displayed a linear increase from the beginning of seed formation to maturation. Several compositions, myristic acid, behenic acid and lignoceric acid, were just found at initial seed filling phase and significantly declined with seed maturity, suggesting that fatty acid composition of castor seed in the initial phases of seed formation differed substantially form that of the mature seeds. The correlation analysis results revealed significant positive correlations of seed development time with mostly fatty acids, further demonstrating that the fatty acids were closely correlated with seed development. These obtained results will benefit for breeding research in high oil content of castor.

Keywords Ricinus communis L.; fatty acids; oil content; seed development; correlation coefficient

Introduction

Castor (Ricinus communis L.) is one of the most important industry oilseed crops, and mainly used industry, medicine and agriculture. Although castor seed is a non-edible, its productions were also widely used as lubricating agent, emulgator, and plasticizer. The fatty acid composition of castor seed has been reported in many papers. Previous researches have demonstrated that the fatty acid composition of castorbean oil by and large includes eight main ingredients, and their content depends on genetic and environmental conditions (Fengjuan et al., 2008; Huang et al., 2015b; Ucciani et al., 2014). It is well known that oil content and fatty acid composition of castor seed are significant different among these reports from different area, suggesting that oil content closely depends in the environmental conditions (Salimon et al., 2010; Bale et al., 2013a; Khan et al., 2010). Furthermore, Lin et al. (1998) and McKeon et al. (2000) have been found the biosynthesis pathway and its key enzymes of castor oil, which was useful for molecular breeding to improving the oil content.

Recently, with nonrenewable resource such as oil and natural gas becoming shortage, castor oil as renewable resource have been attracted great attention in the world. China is the second largest producer of castor. However, the cultivated area is gradually decrease year by year caused castorbean in short supply due to castor low field and price, therefore, increase production is the main objective in castor breeding. The oil high quality breeding research of castor has just begun in recent years. But the following questions is still unknown, including what are the fatty acid composition of castor seed in different development stages, what is the dynamic change of each composition, and how does ricinoleic acid relate to other fatty acids under developmental stages.

Therefore, the main objective of this study was to detect and investigate the fatty acid composition of castor seed.
under different development stage using soxhlet extraction and capillary gas chromatography methods to find the dynamic change of fatty acid composition and their relationships, and these results will benefit for breeding research in high oil content of castor.

1 Results
1.1 Castor seed characteristics under different development stage
In this study Figure 1 gives the morphological observation of seed in different development stage. From 10 d to 40 d of fruit development, castor capsule gradually grown. After 50 d, fruit pericarp became dry and crack. Similar findings were found in seeds. The seed characteristics were listed in Table 1. The seed length varied from 1.12 to 1.52 cm in different development stage, and the highest and lowest lengths were in 40 d and 10 d, respectively. The seed volumes ranged from 1.36 to 2.27 cm$^3$. The weight of 100 seeds and 100 dehusked seeds varied in a large range from 36.84 to 48.62 g, and from 23.67 to 34.03 g, respectively. There was large amplitude of 28.49 g in the weight of 100 dehusked oven-dried seeds.

![Figure 1 Morphological characteristics of castor in different development stages. A: castor fruit; B: castor seed.](image)

| Developmental stage (d) | Length (cm) | Volume (cm$^3$) | 100-seed weight (g) | 100-seed weight (dehusked seeds) (g) | 100-seed weight (dehusked oven-dried seeds) (g) |
|-------------------------|-------------|----------------|---------------------|---------------------------------------|-----------------------------------------------|
| 10                      | 1.12±0.01a  | 1.36±0.01a     | 36.84±0.96a         | 23.67±1.41a                           | 1.98±0.06a                                    |
| 20                      | 1.32±0.01b  | 1.75±0.01b     | 45.01±0.72b         | 26.87±0.21b                           | 9.78±0.14b                                    |
| 30                      | 1.40±0.00c  | 2.02±0.01d     | 47.63±0.93c         | 29.38±0.57c                           | 17.52±0.32c                                   |
| 40                      | 1.52±0.00d  | 2.27±0.01e     | 48.62±0.80c         | 34.03±0.06d                           | 28.16±0.02d                                   |
| 50                      | 1.41±0.01c  | 1.96±0.01c     | 38.65±0.01a         | 30.64±0.02c                           | 28.28±0.03d                                   |
| 60                      | 1.30±0.01b  | 1.75±0.02b     | 38.41±0.04a         | 31.21±0.01c                           | 30.47±0.08e                                   |

1.2 Crude fat content, fatty acid composition of castor seed under different development stage
The crude fat percentage of castor seed in different development stage ranged from 0.25% to 50.69%, while this percentage was higher in the corresponding de-husked seeds, varied from 0.39% to 62.89% (Table 2). In addition, this percentage of dehusked oven-dried seed also has a relatively high range, from 4.69% to 63.91%. All the percentage values of crude fat were the highest in 60-day-old seed. The oil content of castor seed was in the dynamic change along with seed development stage. Oil content reached the highest value at the end of seed development (Table 2). Similarly, this rule was consistent with the findings in cotton (Song et al., 2010),
sunflower (Onemli, 2012), in which oil accumulation started at the beginning of seed development and ended at seed maturation.

Table 2 Crude fat content of castor seed with different development stage

| Developmental stage (d) | Seed Crude fat content (%) | Dehusked Crude fat content (%) | Dehusked oven-dried seed |
|-------------------------|----------------------------|-------------------------------|-------------------------|
| 10                      | 0.25±0.01a                 | 0.39±0.01a                    | 4.69±0.02a              |
| 20                      | 10.23±0.13b                | 17.15±0.37b                   | 47.13±0.61b             |
| 30                      | 22.23±0.04c                | 36.03±0.28c                   | 60.44±0.21c             |
| 40                      | 36.55±0.55d                | 52.19±0.01d                   | 62.62±0.15d             |
| 50                      | 46.04±0.04e                | 58.09±0.02e                   | 62.93±0.05de            |
| 60                      | 50.69±0.44f                | 62.89±0.23f                   | 63.91±0.41e             |

Table 3 Fatty acid composition and percentage of castor seed with different development stage

| Developmental stage (d) | 10 | 20 | 30 | 40 | 50 | 60 |
|-------------------------|----|----|----|----|----|----|
| C°                      | 0.60±0.03c | 0.20±0.03b | 0.0±0a | 0.0±0a | 0±0a | 0±0a |
| Myristic acid S°        | 0.002±0a  | 0.02±0b  | 0±0a | 0±0a | 0±0a | 0±0a |
| A°                      | 0±0a | 0.01±0b | 0±0a | 0±0a | 0±0a | 0±0a |
| Palmitic acid C°        | 24.30±0.23c | 9.50±0.35b | 1.40±0.21b | 1.10±0.03a | 1.20±0.03a | 1.20±0.07a |
| S°                      | 0.06±0a | 0.97±0.04e | 0.30±0.05b | 0.41±0.01c | 0.54±0.01d | 0.59±0.04d |
| A°                      | 0.02±0a | 0.43±0.01d | 0.14±0.02b | 0.19±0.01c | 0.20±0.01c | 0.21±0.02c |
| C°                      | 1.70±0.07b | 1.20±0.01a | 1.20±0.10a | 1.20±0.03a | 1.20±0.01a | 1.20±0.06a |
| Stearic acid S°         | 0.005±0a | 0.12±0b | 0.26±0.02c | 0.43±0.01d | 0.55±0e | 0.61±0.03f |
| A°                      | 0.002±0a | 0.05±0b | 0.12±0.01c | 0.21±0.01d | 0.21±0d | 0.23±0.01d |
| Palmitic acid C°        | 21.80±0.58c | 7.40±0.27b | 4.80±0.24a | 4.60±0.24a | 4.80±0.20a | 4.80±0.49a |
| S°                      | 0.06±0a | 0.76±0.03b | 1.07±0.05b | 1.67±0.08c | 2.19±0.09d | 2.45±0.25d |
| A°                      | 0.02±0a | 0.34±0.01b | 0.51±0.02c | 0.81±0.04d | 0.85±0.04d | 0.94±0.09d |
| C°                      | 31.30±0.15d | 19.10±0.23c | 6.50±0.15b | 5.40±0.01a | 5.40±0.06a | 5.60±0.23a |
| Linoleic acid S°        | 0.08±0.02a | 1.95±0.02c | 1.44±0.03b | 1.97±0.01c | 2.49±0.03d | 2.82±0.12e |
| A°                      | 0.03±0a | 0.88±0.01c | 0.69±0.01ab | 0.96±0d | 0.96±0.01d | 1.08±0.04e |
| C°                      | 10.40±0.17c | 4.10±0.03b | 0.70±0.003a | 0.50±0.03a | 0.50±0.03a | 0.50±0.03a |
| Linoic acid S°          | 0.03±0a | 0.42±0e | 0.15±0.01b | 0.19±0.01c | 0.25±0.02d | 0.27±0.02d |
| Arachidic acid S°       | 0.002±0a | 0.02±0a | 0.01±0.00a | 0.03±0.01a | 0.03±0.01a | 0.03±0.01a |
| A°                      | 0±0a | 0.01±0a | 0.01±0a | 0.01±0a | 0.01±0a | 0.01±0a |
| C°                      | 0.70±0.06b | 0.50±0.07ab | 0.60±0.06ab | 0.50±0.05a | 0.50±0.03a | 0.50±0.06a |
| Arachidonic acid S°     | 0.002±0a | 0.05±0.01a | 0.13±0.01b | 0.18±0.02bc | 0.21±0.02cd | 0.25±0.03ad |
| A°                      | 0±0a | 0.02±0b | 0.06±0.01c | 0.09±0.01d | 0.08±0.01d | 0.10±0.01d |
| Behenic acid S°         | 0.003±0a | 0.02±0b | 0±0a | 0±0a | 0±0a | 0±0a |
| A°                      | 0.001±0b | 0.009±0c | 0±0a | 0±0a | 0±0a | 0±0a |
| C°                      | 0.80±0.06c | 0.20±0b | 0±0a | 0±0a | 0±0a | 0±0a |
| Lignoceric acid S°      | 0.002±0b | 0.02±0c | 0±0a | 0±0a | 0±0a | 0±0a |
| A°                      | 0.001±0b | 0.01±0c | 0±0a | 0±0a | 0±0a | 0±0a |
| C°                      | 6.70±0.06a | 57.00±0.42b | 84.90±1.64c | 86.80±0.22c | 86.50±0.29c | 86.10±0.95c |
| Ricinoleic acid S°      | 0.02±0a | 5.80±0.04b | 18.86±0.36c | 31.71±0.08d | 39.81±0.14e | 43.66±0.48f |
| A°                      | 0.006±0a | 2.62±0.02b | 8.98±0.17c | 15.41±0.04d | 15.39±0.05d | 16.76±0.18e |

Note: 1: crude fatty acid; 2: seed; 3: absolute content; 4: lower case represents significant different at P<0.01.

By using chromatographic analysis, the fatty acid composition of castor seed was detected and included myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, arachidonic acid, behenic

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acid, lignoceric acid and ricinoleic acid (Table 3). However, their contents were pronounced difference in crude fat and seeds. Ricinoleic acid was the greatest change among all fatty acid compositions of seed under different development stage, from 6.70% to 86.80%, followed by linoleic acid (5.60%-31.30%) and palmitic acid (1.20%-24.30%) (Table 3). Interestingly, several compositions were just found at initial seed filling phase and significantly declined with seed maturity, e.g. myristic acid, behenic acid and lignoceric acid were detected in 10-20 day-old seeds, suggesting that fatty acid composition of castor seed in the initial phases of seed formation differed substantially form that of the mature seeds, which were similar to previous studies (Onemli, 2012; Baud and Lepiniec, 2010).

Zeng et al. (2009) detected the fatty acid composition of castor seed collected from Hainan, China, and found that the compositions included eight components, palmitic, stearic, oleic, linoleic, ricinoleic, pentadecanoic, octadecenoic and octadecadienoic acid. Ramos et al. (1984) and Ramanjaneyulu et al. (2013) reported six major ingredients of fatty acid compositions in castor bean from South India. Our previous studies reported eight components in fatty acid composition of castor collected from Inner Mongolia (Huang et al., 2015a; Huang et al., 2015b). All those researches indicated that fatty acid composition and content in different varieties were significant different. Interestingly, in this study, we found that mostly major component in mature seed (30 d-60 d), meanwhile, three other ingredients were firstly detected in earlier development stage of seed, including myristic, behenic and lignoceric acid, which were absence in mature seed, which was also agree with (Takeuchi et al., 1968). And our result suggested that fatty acid composition of castorbean was changed with development stage.

1.3 Correlation analysis and Cluster analysis of fatty acid and seed development time

In this study, the correlation analysis of different fatty acid composition and seed development time was employed to investigate the content of composition under different stage (Table 4). The analysis results revealed significant positive correlations of seed development time with stearic acid, oleic acid, linoleic acid, arachidonic acid and ricinoleic acid, but showed negatively correlated with myristic acid, behenic acid and lignoceric acid, suggesting that the content of stearic acid, linoleic acid and ricinoleic acid increased during the development of seed and indicating that synthesis of fatty acid composition were closely correlated with seed development. Oil content was significantly positive relation with seed development time, stearic acid, oleic acid, arachidonic acid and ricinoleic acid, while negatively correlated with myristic acid, behenic acid and lignoceric acid. Palmitic acid was positive correlation with linolenic acid, behenic acid and lignoceric acid. Linoleic acid showed positive correlation with all others. In addition, a significant positive correlation was observed between oleic acid, arachidonic acid and stearic acid.

The myristic, behenic and lignoceric acid contents were found to associate negatively with developing seed, demonstrating that it was hard to detect those acids in mature seed. The content of linoleic, palmitic and oleic acid decreased during castor seed development, while opposite result was found in Song et al. (2010). Additionally, Rebetzke et al. (1996) revealed negative correlation between palmitic and oleic acid in soybean, which was inconsistent with our present work. This different relationship may be due to species-specific factors.

Cluster analysis for fatty acid compositions and content is shown in Figure 2. Based on these characteristics, six different developmental stages of seeds were classified into two groups. Group I included seed under 40-, 50- and 60-day-old. Ten to thirty-day-old seed were separately joined together. This grouping result reflected the fatty acid composition of castorbean seed. Group I included mostly main fatty acid, which also found in other study (Bale et al., 2013) and other crops (Guo et al., 2015; Choudhary et al., 2015). Group II mainly included trace amount of myristic, behenic and lignoceric acid. The findings clearly suggested that fatty acid compositions could alter in the seed development, in which associated with fatty acid metabolism related enzyme activity (Zhong-min et al., 2011).
Table 4 Correlation analysis of fatty acids and seed development time of castor seed

| Development time | Mytistic acid | Palmitic acid | Stearic acid | Oleic acid | Linoleic acid | Linolenic acid | Arachidic acid | Behenic acid | Lignoceric acid | Ricinoleic acid |
|------------------|--------------|---------------|--------------|------------|---------------|----------------|----------------|--------------|----------------|----------------|
| Mytistic acid    | -0.39        |               |              |            |               |                |                |              |                |                |
| Palmitic acid    | 0.16         | 0.83*         |              |            |               |                |                |              |                |                |
| Stearic acid     | 0.96**       | -0.45         | 0.12         |            |               |                |                |              |                |                |
| Oleic acid       | 0.97**       | -0.33         | 0.24         | 0.99**     |               |                |                |              |                |                |
| Linoleic acid    | 0.80*        | 0.14          | 0.66         | 0.81*      | 0.88**        |                |                |              |                |                |
| Linolenic acid   | 0.24         | 0.78*         | 1.00**       | 0.2        | 0.32          | 0.73           |                |              |                |                |
| Arachidic acid   | 0.65         | 0.2           | 0.66         | 0.69       | 0.77*         | 0.94**         | 0.72           |              |                |                |
| Arachidonic acid | 0.94**       | -0.47         | 0.09         | 0.99**     | 0.98**        | 0.80*          | 0.18           | 0.71         |                |                |
| Behenic acid     | -0.47        | 0.99**        | 0.77*        | -0.53      | -0.42         | 0.04           | 0.71           | 0.09         | -0.56          |                |
| Lignoceric acid  | -0.47        | 0.99**        | 0.78*        | -0.52      | -0.41         | 0.05           | 0.72           | 0.1          | -0.55          | 1.00**         |
| Ricinoleic acid  | 0.95**       | -0.49         | 0.06         | 1.00**     | 0.98**        | 0.78*          | 0.15           | 0.67         | 0.99**         | -0.58          |
| Oil content      | 0.99**       | -0.42         | 0.14         | 0.98**     | 0.98**        | 0.81*          | 0.21           | 0.67         | 0.96**         | -0.51          |

Note: *Significant at $P < 0.05$

**Significant at $P < 0.01$
2 Conclusion

Here, we firstly analyzed the fatty acid composition in castor seed development and their relationships, and the results suggested that the composition were closely correlated with seed development. The findings of this work will be useful for designing management practices to obtain a specific oil quality and in improving the predictions of crop models.

3 Materials and Methods

3.1 Plant materials

The castor seed (2129 castor accession) was collected from Academy of Agricultural Science in Tongliao, China. The mature and healthy seeds were grown in farmland of Inner Mongolia University for the Nationalities, Tongliao, which belongs to the continental monsoon climate. In this district, the annual precipitation ranges from 350mm to 450mm, the annual effective windy time (the wind speed is about 3m/s to 20m/s) varies from 5000h to 6000h and the annual effective wind power density changes from 100 to 150 W/m² (He Junyan, 2011). The fruits were harvested at 10, 20, 30, 40, 50 and 60 days after anthesis (Figure 1). The seeds were extracted immediately after harvest for next experiments (Figure 1).

3.2 Measurement of relatively parameters of seed under different developmental stages

The length (L, longest axis) of each seed population were measured on 10 randomly selected seeds under different development stage using a digital vernier caliper (Guanglu 0-200, China) with a reading accuracy within 0.01 mm. Seed volume was counted based on displaced water, which is collected and weighed is used to calculate equivalent volume of water. One hundred seeds (digital electronic balance, CPA225D) were also weighed by means of an electronic balance reading to 0.001 g. Husk from each seed was carefully removed and then hundred husk-seed mass was determined. Seeds were dried in an oven at 60±2°C for 3 h. One hundred weights of de-husked and dried seeds were measured. Values obtained for weights, lengths and volume of all seeds in each replicate were averaged. Seeds in different development stage were selected for morphological observation using stereoscopic microscope (XTL-7046SZ, COSSIM, 7×).

3.3 Measurement of fatty acid composition and content

The crude fatty acid content of seed with different development stage was determined with three replications using soxhlet extraction method according to Chemist and Horwitz (1980). The fatty acid composition and content were measured according to our previous report (Huang et al., 2015b; Huang et al., 2015a). The fatty acid composition ratio was calculated based on the corresponding chromatographic peaks. The character and structure of fatty acid compositions in standard sample and each seed were distinguished using the total ion current (TIC) of mass spectrometer (GC5400, Skyray Instrument, China) (Figure 3). By applying the computer automatic and manual retrieval with NIST98 and Wiley of Mass Spectral Data (Ausloos et al., 1992; McLafferty and Stauffer, 1994), fatty acid composition during different development stages of seed was measured and analyzed.

3.4 Data analysis

All data were evaluated using Statistics 17.0 software and the results of the test performed are given with the
mean value ± standard deviation (SD). Single factor-analysis of variance (ANOVA) for fatty acid composition and content in relation to seed size were calculated. Significance of difference and Correlation of seed characteristics, i.e. mean seed weight (dry seeds without husk) and fatty acid composition (%) was calculated.

Figure 3 Representative chromatogram of fatty acid methyl ester in castor bean. A: standard substance; B: ten-day-old seed.

Authors’ contributions
Xiaofeng Chen and Mu Peng carried out the studies and drafted the manuscript. Fenglan Huang, Rui Luo and Yong Zhao participated in the design of the study and performed the statistical analysis. Chunguang Bao, Xue Lei and Yue Li conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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