

## Analysis of Nutritional Components and Extracts in *Castanopsis hystrix* Seeds

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This study investigated the nutritional components of *Castanopsis hystrix* seeds and evaluated their potential commercial value, regarding multi-function management of *C. hystrix* plantations. The following nutritional components of *Castanopsis hystrix* seeds were determined according to national standards: starch, fat, protein, reducing sugars, and amino acids. The chemical composition of their 50% benzene-alcohol(v/v) extracts was analyzed by GC-MS. Results showed that the contents of water, starch, fat, protein, and reducing sugar in the seeds were 36.08%, 68.11 g/100g, 0.3 g/100g, 3.98 g/100g, and 0.75 g/100g, respectively. The total amino acid content in the seeds is 3.42 g/100g, containing 16 kinds of amino acids, with 8 essential amino acids, which amount to 1.09g/100g. The amino acid SRC value was 84.02, indicating high nutritional value. A total of 26 compounds were identified in the extract of these seeds, with the highest content of aldehydes. The main compounds were 5-hydroxymethylfurfural (23.37%), melezitose (15.88%), palmitic acid (9.04%), and stearic acid (5.23%). The above analysis indicates that *Castanopsis hystrix* seeds have high nutritional value, as well as potential antioxidant and anti-tumor properties, which may have the potential to be used in food and medicine fields, with broad application prospects.

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### INTRODUCTION

*Castanopsis hystrix*, Fagaceae is an evergreen broad-leaved tree with wide growth adaptability, fast growth rate, beautiful wood texture, and rosy wood color. It is an ideal material for furniture, handicrafts, and vehicles (Lü et al. 2006; Diao et al. 2015; Gao et al. 2022; Li et al. 2022). *Castanopsis hystrix* is a precious timber tree species that has been popularized in the southern subtropical region of China. It has been planted in large areas in Guangdong, Guangxi, Fujian, Yunnan and other places. Only Guangxi has its planting area exceeding 130,000 hm<sup>2</sup> (Wang et al. 2022; Liu et al. 2023). However, *C. hystrix* wood usually takes 30 to 50 years to mature, resulting in low economic benefits of *C. hystrix* plantation management.

*Castanopsis hystrix* grows fast and is rich in unused resources, such as seeds and leaves. From July to August every year, the understory of *C. hystrix* plantations is covered

with a layer of seeds, which are picked up by locals and eaten directly, but the nutritional content of *C. hystrix* seeds is unknown. The seeds of many tree species are rich in nutrients, secondary metabolites and other biological activities, which can be used as raw materials for food, food additives and medicines, and have high economic value, such as *Dalbergia odorifera* T. Chen, *Torreya grandis*, *Castanea henryi* (Skam) Rehd. et Wils, etc (Shokoofeh *et al.* 2018; Wang *et al.* 2018; Wei *et al.* 2018; Ma *et al.* 2020). *Castanea henryi* (Skam) Rehd. et Wils. is a member of Fagaceae, sharing the same family as *Castanopsis hystrix*. Its seeds are rich in nutrients and are one of China's important woody food crops (Fan *et al.* 2017).

The present study investigated the nutritional contents of starch, fat, reducing sugar and other nutrients in *Castanopsis hystrix* seeds to fully understand their economic value and to provide a reference for, 1.) development and utilization of *C. hystrix* seeds, and 2.) multi-function management for a *Castanopsis hystrix* plantation.

## EXPERIMENTAL

### Test Material

*Castanopsis hystrix* seeds were collected from the Shaoping Experimental Field of the Experimental Forest Center of the Chinese Academy of Forestry in Pingxiang City, Guangxi. They are naturally shedding seeds. After collection, the seeds were stored at 4 °C.



Fig. 1. *Castanopsis hystrix* seed

### Sample Processing

*Castanopsis hystrix* seeds were peeled and mouldy seeds were removed, ground into powder using a shredder, and sieved on a 40- to 60-mesh screen.

### Determination of Basic Components

The determination of seed moisture content, protein content, and fat content were determined by Zhang *et al.* (2016). The moisture was determined by placing the seeds in an oven (101-1B, Lichen Scientific) at 103 °C until they reached a constant weight; the protein content was determined using the Kjeldahl method, in which the protein was calculated using a nitrogen conversion factor of 6.25; the fat content was determined using the acid hydrolysis method.

Naturally present reducing sugars in *C. hystrix* seeds were determined by direct titration of ground seeds samples (without acid hydrolysis) with Fehling's method (Yang *et al.* 2015). Starch content was measured by an acid hydrolysis method. Sample starch was hydrolyzed to reducing sugars with HCL after removal of fat and soluble sugars, then

reducing sugars content was determined by direct titration method, and finally reducing sugars content was converted into starch content (Yang *et al.* 2019).

### Amino Acid Composition Analysis

Amino acids were assessed by an L-8900 automatic amino acid analyzer, as previously described, with some modification (Chen *et al.* 2023). Dry sample powder, 0.4 g, was added to 15 mL 6 M hydrochloric acid at 110 °C for 22 h. The volume was adjusted to 50 mL with double-distilled water. Then, 1 mL extraction was used for rotary evaporation and then reconstituted with 1 mL sodium citrate buffer. The solution to be detected was then filtered through a 0.22 µm filter. The peak time and peak area of the standard were used to calculate the various amino acid contents.

### Chemical Composition Analysis of Extracts

A total of 2.00 g of seed powder was weighed, added to 10 mL of 50% benzene-alcohol(v/v) solution, and the mixture was ultrasonically extracted in ice water for 1 h, and then the upper liquid was taken out and let stand for 10 min. The extraction was repeated for 3 times. The upper liquid was combined, the solution was evaporated to dryness, and 5 mL of methanol solution was added to dissolve the extract, which was then determined using a triple quadrupole-gas chromatograph-mass spectrometer (TRACE 1300-TSQ 9000, Thermo Fisher Scientific).

Mass spectrometry conditions were as follows: Scanning mode: full scan; Scanning mass range: 30 to 700 m/z; Ion source: EI; Ion source temperature: 300 °C; Injection mode: splitless; Injection port temperature: 280 °C; carrier gas: high purity helium; Solvent delay time: 3.5 min; Chromatographic conditions: chromatographic column, TG-5SILMS (30 m × 0.25 mm × 0.25 µm); Column flow rate: 1.0 mL/min; Injection volume: 1 µL; Temperature programming: 40 °C for 3 min, then increased to 90 °C at 3 °C/min, and then increased to 220 °C at 10 °C/min for 10 min. Data was processed using Excel software.

## RESULTS AND ANALYSIS

### Contents of Basic Nutrients in *Castanopsis hystrix* Seeds

The basic nutrients and contents of *Castanopsis hystrix* seeds are shown in Table 1. The highest content of starch in *Castanopsis hystrix* seeds was 68.1 g /100 g, which was similar to that of *Castanea henryi* ‘Youzhen’ (71.126 g/100 g) and ‘Chushuhong’ (71.351 g /100g) (Yang 2014), and higher than that of *Castanea mollissima* (49.02 to 55.13 g/100 g) (Zhang *et al.* 2016). Higher starch content indicates that it has the potential to be used as a starch food raw material. The fat content of *C. hystrix* seeds is 0.3 g/100 g, which is lower than that of nuts such as walnuts, almonds, and cashews (Cui *et al.* 2023), and is similar to that of chestnut (Zhang *et al.* 2016). Chestnut is a healthy nut food with low fat content, indicating that *C. hystrix* seeds can also be eaten as a healthy nut food (Yang *et al.* 2015). The seed protein content of *C. hystrix* was 3.98 g/100 g, which was higher than that of *Cyclobalanopsis gracilis*, *Cyclobalanopsis glauca*, *Castanopsis sclerophylla*, *Castanopsis tibetana* (Liu *et al.* 2009) and *Castanea henryi* ‘Huali 3<sup>rd</sup>’, and close to ‘Huali 2<sup>nd</sup>’ (He *et al.* 2022). The metabolism of the seeds did not stop during the storage at 4 °C. Part of the protein was decomposed to provide the energy required for physiological metabolic activity, and the protein content decreased with the increase of storage days (Tang *et al.* 2006). Therefore, the protein content of fresh *C. hystrix* seeds should be higher

than 3.98 g/100 g. The content of reducing sugar in *C. hystrix* seeds was 0.75 g/100 g, which was lower than that in *C. mollissima* (Zhang *et al.* 2016).

**Table 1.** Basic Nutritional Components in *Castanopsis hystrix* Seeds

Nutritional Components	Contents
Water (%)	36.08 ± 0.35
Starch (g·100g <sup>-1</sup> )	68.11 ± 0.29
Fat (g·100g <sup>-1</sup> )	0.3 ± 0.004
Protein (g·100g <sup>-1</sup> )	3.98 ± 0.15
Reducing Sugar (g·100g <sup>-1</sup> )	0.75 ± 0.009

### Analysis of Amino Acid Content in *Castanopsis hystrix* Seeds

Amino acids are closely related to the life activities of organisms and are essential nutrients for humans. The amino acid composition of *Castanopsis hystrix* seeds is shown in Table 2. A total of 16 amino acids were detected in the seeds of *C. hystrix*, including 8 essential amino acids (1.09 g/100 g) and 8 non-essential amino acids (2.33 g/100 g). The total amino acid content was 3.42 g/100 g, which was higher than that of *C. mollissima*, found in a previous study (2.56 to 3.27 g/100 g) (Zhang *et al.* 2016).

**Table 2.** Contents of Amino Acids in *Castanopsis hystrix* Seeds

Amino Acids	Contents (g·100g <sup>-1</sup> )
Asp	0.61
*Thr	0.13
Ser	0.14
Glu	0.70
Pro	0.06
Gly	0.15
Ala	0.23
*Val	0.16
*Met	0.07
*Ile	0.12
*Leu	0.22
*Tyr	0.09
*Phe	0.12
His	0.09
*Lys	0.18
Arg	0.35
EAA	1.09
NEAA	2.33
TAA	3.42
EAA/TAA	32%

Note: \*Essential amino acids

The content of leucine in the essential amino acids of *C. hystrix* seeds is the highest, which is 0.22 g/100 g. Leucine is an essential amino acid for the synthesis of plasma protein and tissue protein. In addition, it can also participate in the synthesis of skeletal muscle protein and inhibit the degradation of skeletal muscle protein, which is an important amino acid in human body (Wang *et al.* 2014). The content of glutamic acid in non-essential amino acids of *C. hystrix* seeds is the highest, which is 0.7 g/100 g. Glutamic acid can regulate the immune function of human body and participate in various metabolic activities

in human body (Su *et al.* 2016). The EAA / TAA and EAA / NEAA values of *C. hystrix* seeds were 32% and 47%, respectively, which were somewhat different from the FAO / WHO ideal model of EAA / TAA values of 40% and EAA / NEAA values of 60%. However, the EAA / TAA and EAA / NEAA values of *C. hystrix* seeds were higher than walnuts, almonds, hazelnuts, and Hawaiian nuts (Cui *et al.* 2023).

The closer the proportion of essential amino acids in food to the needs of the human body, the higher the nutritional value. The nutritional value of amino acids in *Castanopsis hystrix* seeds was evaluated by amino acid ratio coefficient method (Zhu and Wu 1988), and the results are shown in Table 3. If the food amino acid ratio coefficient (RC value) is 1, the amino acid composition of the food is consistent with the amino acid composition in the WHO/FAO ideal model. When the RC value is greater than 1, this indicates that the amino acid is too much, and a RC value that is less than 1 indicates the amino acid is too little. The RC values of threonine, valine, leucine, lysine, and isoleucine in *C. hystrix* seeds were close to 1, indicating that the content of such amino acids was ideal. The RC value of phenylalanine + tyrosine was greater than 1, indicating that the amino acid content was relatively excessive. The RC value of methionine was less than 1, indicating that the amino acid content was relatively insufficient, and the RC value of methionine was the lowest, which was the first limiting amino acid of *C. hystrix* seeds. Amino acid ratio coefficient score (SRC) represents the dispersion degree of amino acid RC value, and the larger the value is, the higher is the amino acid nutritional value of the food. The SRC of amino acids in *C. hystrix* seeds was 84.02, which was significantly higher than walnut, almond, pecan, cashew nut, hazelnut, *Macadamia ternifolia*, and other nut foods (Cui *et al.* 2023), and also higher than *Macaranga adenantha* and *Mesua ferrea* seeds (Li *et al.* 2023), indicating that the amino acids in *C. hystrix* seeds have high nutritional value.

**Table 3.** RAA, RC, and SRC of Amino Acids in *Castanopsis hystrix* Seeds

Amino Acids	EAAV/%		RAA	RC
	Sample	WHO/FAO		
Thr	3.80	4	0.95	1.06
Val	4.68	5	0.94	1.05
Met	2.05	3.5	0.58	0.66
Ile	3.51	4	0.88	0.98
Leu	6.43	7	0.92	1.03
Phe+Tyr	6.14	6	1.02	1.15
Lyu	5.26	5.5	0.96	1.07
SRC	84.02			

### Analysis of Components of Seed Extract of *Castanopsis hystrix*

The components of the seed extract of *Castanopsis hystrix* were analyzed by GC-MS technique, the compounds were qualitatively characterized by computer spectrum library (NIST17. L), and the relative percentage content of each compound was calculated by area normalization method. As shown in Table 4, a total of 26 compounds, accounting for 89.82% of the total peak area, were identified from the seed extract of *C. rubra*. In the extract, aldehydes accounted for 26.03%, ketones accounted for 9.05%, alcohols accounted for 23.25%, esters accounted for 6.17%, alkenes accounted for 3.7%, fatty acids accounted for 16.73%, and other substances accounted for 4.89%. The content of aldehydes was the highest, followed by alcohols, and fatty acids also had a higher content.

The component with the highest content in the extract of *Castanopsis hystrix* seeds was 5-hydroxymethylfurfural, which had strong antioxidant activity (Okutsu *et al.* 2015),

which indicated that *C. hystrix* seeds had certain antioxidant activity. Isosorbide dinitrate has certain effects on cardiovascular and cerebrovascular diseases (Wang *et al.* 2017). Three fatty acids were detected in the extract of *C. hystrix* seeds, namely palmitic acid (9.04%), linoleic acid (2.46%), and stearic acid (5.23%). Among them, palmitic acid can induce apoptosis of insulinoma cells and has anti-tumor effect (Liang *et al.* 2013; Hsiao *et al.* 2014; Wang *et al.* 2023). Linoleic acid cannot be synthesized in human body. It is an essential unsaturated fatty acid, which can improve immunity and promote metabolism (Ji *et al.* 2013).

**Table 4.** Composition and Relative Content of Extracts *Castanopsis hystrix* Seeds

No.	Retention Time	Name	CAS	relative content
1	3.23	Acetol	116-09-6	0.89%
2	3.62	Methyl glycolate	96-35-5	2.56%
3	4.08	Glycerol	56-81-5	0.34%
4	4.21	Methyl ( $\pm$ )-lactate	2155-30-8	0.99%
5	4.56	Diepoxybutane	1464-53-5	0.84%
6	6.57	3-Furaldehyde	498-60-2	2.66%
7	7.74	Furfuryl alcohol	98-00-0	1.11%
8	10.01	1,3-Dihydroxyacetone	96-26-4	3.23%
9	12.34	Acetylformoin	10230-62-3	1.15%
10	16.68	DL-Arabinose	20235-19-2	0.75%
11	17.76	Tetradecane	629-59-4	0.54%
12	20.42	Spermine tetrahydrochloride	28564-83-2	3.78%
13	22.21	$\beta$ -Lactose	5965-66-2	1.47%
14	22.60	Isosorbide dinitrate	87-33-2	2.72%
15	23.29	5-Hydroxymethylfurfural	67-47-0	23.37%
16	24.65	D-Mannose	3458-28-4	1.00%
17	24.76	Desulfosinigrin	5115-81-1	0.79%
18	25.06	Methyl 6-oxoheptanoate	2046-21-1	0.89%
19	26.86	Paromomycin	7542-37-2	0.77%
20	27.64	2,4-Di-t-butylphenol	96-76-4	1.80%
21	27.81	Maltose	69-79-4	2.70%
22	29.79	MelezitoseMelezitose	597-12-6	15.88%
23	32.68	Palmitic acid	57-10-3	9.04%
24	34.63	Linoleic acid	60-33-3	2.46%
25	34.69	(Z)-13-octadecenoic acid	693-71-0	2.86%
26	35.04	Stearic acid	57-11-4	5.23%

## CONCLUSIONS

1. The contents of moisture, starch, fat, protein, and reducing sugar in *Castanopsis hystrix* seeds were 36.08%, 68.11 g/100 g, 0.3 g/100 g, 3.98 g/100 g and 0.75 g/100 g, respectively. *Castanopsis hystrix* seeds had higher starch content, indicating that it could be used as a raw material for starchy foods. The protein content was higher than that of *Castanea mollissima* (Zhang *et al.* 2016), and the fat and reducing sugar contents were lower. *Castanopsis hystrix* seeds are a kind of healthy nut food.
2. A total of 16 kinds of amino acids were detected in the seeds of *Castanopsis hystrix*. The total amino acid content and essential amino acid content were 3.42 g/100 g and 1.09 g/100 g, respectively. The amino acid SRC value was 84.02, which was higher than that of various nuts and had high amino acid nutritional value.
3. A total of 26 compounds were identified in the extracts of seeds of *Castanopsis hystrix*, accounting for 89.82% of the total area of the peaks. The content of aldehydes was the highest, followed by alcohols, fatty acids, ketones, esters and alkenes. The main component of *Castanopsis hystrix* was 5-hydroxymethylfurfural, which had strong antioxidant activity. Isosorbide dinitrate, palmitic acid, and linoleic acid also had certain biological activities.
4. The cultivation period of *Castanopsis hystrix* is long, and the economic benefit of its plantation management is low. However, *C. hystrix* has abundant seed resources. Making full use of its seed resources can improve the value of *C. hystrix* and promote the multi-functional management of *C. hystrix* plantations. This study shows that *C. hystrix* seeds have high nutritional value, and have potential antioxidant, anti-tumor, and other biological activities. Therefore, *Castanopsis hystrix* seeds have broad application prospects.

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