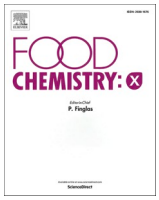
Food Chemistry: X 23 (2024) 101770

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



# Effects of color protection and enzymatic hydrolysis on the microstructure, digestibility, solubility and swelling degree of chestnut flour

Wenxin Guo [a,b], Liyang Yang [a,b], Xinyu Shi [a,b], Xin Cong [a,b], Shuiyuan Cheng [a,b], Linling Li [a,b,*], Hua Cheng [a,b,*]

[a] School of Modern Industry for Selenium Science and Engineering, Wuhan Polytechnic University, Wuhan 430048, China
[b] National R&D Center for Se-Rich Agricultural Products Processing, Wuhan Polytechnic University, Wuhan 430023, China

ARTICLE INFO

*Keywords:*
Chestnut flour
Enzymolysis
Digestive properties
Slowly digestible starch
Resistant starch

ABSTRACT

Chestnuts, despite their nutritional value, pose challenges in starch processing, digestion, and absorption. This study employed various color-fixing formulations and processing methods to simulate the *in vitro* digestion of both untreated and enzymatically hydrolyzed chestnut flour. Changes in starch properties, digestion characteristics, and estimated glycemic index (eGI) were analyzed to understand how enzymatic hydrolysis affects chestnut flour properties. The results showed that the browning of chestnut flour was the least when the mass ratio of vitamin C, citric acid, and EDTA-$Na_2$ was 9:1:0.3. Following treatment with pullulanase and glucoamylase, the content of rapidly digestible starch decreased to 10 %, while the content of slowly digestible starch and resistant starch increased to 62 % and 27 %, respectively. The eGI value of chestnut flour after enzymatic hydrolysis increased to 61.85–65.14, the hydrolysis rate was 78.37 %–89.20 %, the water holding capacity was 5.3–8.6, the solubility was 51.33 %–58.33 %, and the swelling degree decreased to 2.21–3.33 mL/g.

## 1. Introduction

*Castanea mollissima* Blume, belonging to the Fagaceae family, is an exceptional woody crop that offers high nutritional value and plays a vital role in both economy and ecology (Cheng et al., 2022). According to the data released by the Food and Agriculture Organization of the United Nations (FAO) in 2019, the global chestnut planting area reached 926,037 ha, with Asia being the largest producer (43.1 %), followed by Europe (14.3 %) and the United States (6.6 %). Chestnuts, which possess considerable economic value, play a significant role in both daily life and research. Starch was the main component of chestnut, which determines the processing characteristics and nutritional function of chestnut flour and its products. Chestnut flour contains high resistant starch (RS), which is beneficial to improve the diet of people with high blood sugar (Hao, Li, Bao, Wu, & Ouyang, 2018; Zhu et al., 2019). Chestnut flour, apart from its rich starch and sucrose content, is also a significant source of high-quality essential amino acids (ranging from 4 % to 8 %), dietary fiber (4 % to 10 %), and unsaturated fatty acids, including oleic acid, linoleic acid, and palmitic acid. Furthermore, chestnut flour is abundantly in vitamin E and C, as well as minerals such as potassium, phosphorus, and magnesium. Additionally, it contains phenolic compounds, which further contribute to its nutritional value and potential health benefits (Li et al., 2022; Zeng, Wang, Chen, & Zheng, 2024). In recent years, the utilization of chestnut flour has become prevalent in the food industry, finding its way into bread, potato chips, biscuits, and various other food products. The inclusion of chestnut flour not only enhances the flavor profile of these foods but also boosts their dietary fiber content, ultimately elevating their nutritional value (Wang, Shi, Chen, Dong, & Chen, 2023).

However, recent studies have demonstrated that the incorporation of chestnut flour accelerates the aging process of bread, resulting in a reduction of its volume and an increase in the size of crumb holes (Wang, Shi, et al., 2023). Additionally, this addition promotes browning, potentially altering the sensory perception of the food. Furthermore, the abundant RS content in chestnuts may pose a challenge for individuals with weaker digestive systems, such as the elderly and young children. According to the digestion resistance of starch, it can be divided into rapidly digestible starch (RDS), slowly digestible starch (SDS), and RS (Englyst, Kingman, & Cummings, 1992). The glycemic index (GI) was used to estimate the relative ability of sugary foods to increase blood sugar levels. Based on their GI value, foods can be classified into three categories: low GI foods (GI $\leq$ 55), medium GI foods (56 $\leq$ GI $\leq$ 69), and

* Corresponding authors at: School of Modern Industry for Selenium Science and Engineering, Wuhan Polytechnic University, Wuhan 430048, China.
*E-mail addresses:* 12316@whpu.edu.cn (S. Cheng), 12622@whpu.edu.cn (L. Li), 12621@whpu.edu.cn (H. Cheng).

https://doi.org/10.1016/j.fochx.2024.101770
Received 19 July 2023; Received in revised form 13 July 2024; Accepted 23 August 2024
Available online 25 August 2024

high GI foods (GI ≥ 70) (FAO/WHO) (Trinidad, Mallillin, Sagum, & Encabo, 2010). High glycemic index (GI) foods have the potential to cause significant fluctuations in blood sugar levels, whereas low GI foods can effectively assist in the regulation of postprandial blood sugar levels (Hao et al., 2018). The percentage of starch in chestnut fruit is 36.61 %. The content of amylose in chestnut starch is 21.86 %. The proportion of RS content in chestnut fruit to starch is 55.64 %. Long term storage will increase the content of RS. Foods with high SDS, RS content, and low GI have potential benefits for chronic diseases (such as cardiovascular diseases) and hyperglycemia (Hao et al., 2018). The physical and enzymatic modification methods of starch were considered environmentally friendly approaches for starch modification (Park et al., 2018). From the perspectives of the environment and food consumers, enzymatic modification of starch is safer and healthier than chemical modification. In addition, enzymatic reactions are highly specific for starch substrates present in complex food matrices (Ashogbon, 2021). During the processing of chestnut flour, enzymatic hydrolysis has been observed to substantially elevate the RS content of instant chestnut flour. However, due to the synergistic interaction between the reduction of sugars and starch hydrolysis, this process also results in an increase in the estimated glycemic index (eGI) of the instant powder (Yang, Wu, & Ouyang, 2023). Additionally, A study illustrated that modified chestnut starch could help attenuate diet-induced obesity *via* a short-chain fatty acids receptor by modulating the expression of some genes in cecal epithemicrobiota, and also showed a positive influence on adjusting gut microbes (Lee, Song, Nam, Nam, Kim, Lee et al., 2020).

Within the current landscape where meal replacements have gained significant popularity as a healthy dietary alternative, this study endeavors to comparatively evaluate the hydrolysis profile, digestion properties, and GI of native and enzymatically hydrolyzed chestnut flour under simulated *in vitro* digestion conditions. The primary objective is to optimize the processing and digestibility of chestnut flour, aiming for a moderate GI value, improved digestibility, and desirable color characteristics.

## 2. Materials and methods

### 2.1. Processing of chestnut materials

Fresh chestnut (*C. mollissima* Blume) of the "Meigui Red" variety were harvested from Luotian, Hubei Province, China, on September 20th, 2022. The starch composition of this specific variety of chestnuts comprises approximately 63.21 % of its dry weight. They were subsequently stored at 4 °C for next use.

For the experiment, fresh chestnuts were selected and their shells were removed either manually or using stainless steel cutting tools. The skin and seed coat were then removed by soaking the chestnuts in hot water at 85 °C for 5 min. Subsequently, the chestnuts were quickly sliced into sections approximately 3 mm thick. These slices underwent color protection treatment by soaking them in the appropriate solution for 10 min. The chestnuts were then ripened, dried and ground using a grinder. The resulting flour was sieved through an 80-mesh sieve to obtain the original chestnut flour.

### 2.2. Chemical and reagents

Pullulanase (≥1000 npun/g, P299007), glucoamylase (≥260 U/mL, A298984), and α-amylase (≥50 U/mL, A109182) were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Additionally, the Plant Starch Content Kit (A148–1-1) and Glucose Kit (Glucose oxidase method, A154–1-1) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All reagents of analytical grade were acquired from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

**Table 1**
Different treatments of chestnut in the study.

| Sample name | Heat treatment | Drying process | Enzymatic treatment |
|---|---|---|---|
| WR-HAD | Whole raw | Hot air drying | No |
| SC-HAD | Semi cooked | Hot air drying | No |
| FC-HAD | Fully cooked | Hot air drying | No |
| WR-FD | Whole raw | Freeze drying | No |
| SC-FD | Semi cooked | Freeze drying | No |
| FC-FD | Fully cooked | Freeze drying | No |
| FH-ER1 | Fully cooked | Hot air drying | Single enzyme pullulanase hydrolysis |
| FH-ER2 | Fully cooked | Hot air drying | α- Amylase and glucoamylase enzymolysis |
| FH-ER3 | Fully cooked | Hot air drying | Pullulanase and glucoamylase enzymolysis |
| WF-ER1 | Whole raw | Freeze drying | Single enzyme pullulanase hydrolysis |
| WF-ER2 | Whole raw | Freeze drying | α- Amylase and glucoamylase enzymolysis |
| WF-ER3 | Whole raw | Freeze drying | Pullulanase and glucoamylase enzymolysis |

### 2.3. Pretreatment and color protection of chestnut flour

In selecting color fixatives, reference was made to the choices of vitamin C (Vc), citric acid, and EDTA-$Na_2$ made by Cheng et al. (Cheng et al., 2023). Base on this, a single-factor experiment was conducted to prepare solutions with varying concentrations. Specifically, Vc solution were prepared with mass fraction of 0.1 %, 0.5 %, and 0.9 %, while 0.5 % citric acid solutions were also prepared. Furthermore, citric acid solutions were formulated with mass fractions of 0.1 %, 0.5 %, and 0.9 %, along with 0.5 % Vc solution and 0.03 % EDTA-$Na_2$ solution. Additionally, EDTA-$Na_2$ solution were prepared with mass fractions 0.01 %, 0.03 %, and 0.05 %, along with 0.5 % Vc solution and 0.5 % citric acid solution. As a control, chestnut slices were not treated with color protection. The duration of color protection was set at 10 min, followed by washing with distilled water. The optimal ratio of color protection solution was determined through a single-factor experiment, and the effectiveness of color protection was evaluated based on the browning index (ΔE).

The color of chestnut flour was determined using a color difference meter. The measurements were repeated three times to obtain an average value. The total color difference (ΔE) between chestnut flour subjected to color-protected drying treatment and non-color-protected drying treatment was determined using the Commission Internationale de l'Eclairage (CIE-LAB) color system. The color difference was calculated according to the following formula,

$$\Delta E = \sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2}$$

This measurement was based on the brightness value (L*), red-green value (a*), and yellow-blue value (b*) of the dried chestnuts. The corresponding values for fresh chestnuts were denoted as $L_0$, $a_0$, and $b_0$, respectively. The ΔE value was calculated using the formula provided, where a higher ΔE indicates a lighter browning degree.

### 2.4. Process optimization of raw chestnut flour

Base on the optimal color protection scheme established earlier, various drying pretreatments and drying methods were implemented (Table 1). Pretreatment methods included full raw (direct drying after color protection treatment without further processing), half cooked

(color protection treatment followed by blanching at 95 °C for 5 min), and fully cooked (color protection treatment followed by cooking at 95 °C for 10 min). For drying methods, hot air drying was conducted at 70 °C for a preset duration of 180 min, terminating when the mass became constant. Alternatively, vacuum freeze-drying treatment involved pre-freezing the cut fragments for 8 h before freeze-drying at −50 °C for 48 h.

### 2.5. Enzymatic hydrolysis of chestnut flour

The enzymatic hydrolysis of chestnut flour followed a standardized technological process. Initially, a starch milk solution was prepared by combining 10 g of the flour with 100 mL of pH 5.8 phosphate buffer solution. This mixture was then stirred and preheated for 30 min. Subsequently, enzymatic hydrolysis was performed at 55 °C for 20 min. Following hydrolysis, the mixture was inactivated in a boiling water bath for 30 min and then cooled down. Centrifugation at 4000 r/min for 8 min separated the enzymatic hydrolysis products from the supernatant. The precipitate was washed three times with water, dried, and crushed through an 80-mesh sieve to obtain the final enzymatic hydrolysis product of chestnut flour. The specific enzyme concentration and formula were referenced from the reagent manual. Table 1 outlines the various combinations of pretreatment and enzymatic treatment employed in this study.

### 2.6. Determination of starch content

The determination of starch involves the conversion of starch into glucose through acidolysis. This glucose was quantified using anthrone colorimetry, enabling the calculation of the corresponding mass fraction of starch. Specific instructions can find in the plant starch content Kit (A148–1-1).

For starch digestion and glucose determination, we followed the protocol of Eyinla et al. (Eyinla, Sanusi, & Maziya-Dixon, 2021), with minor modifications. Briefly, each test tube contained a sample of 100 ± 5 mg and 4 mL of 0.5 mol/L sodium acetate buffer (pH 5.2). These were incubated in an oscillating water at 37 °C for specified time intervals: 0, 10, 20, 30, 60, 90, 120, 150, and 180 min. At each interval, aliquots of 0.1 mL were collected and mixed with 1 mL of 80 % ethanol. The aliquots were centrifuged at 1500 *g* for 2 min to separate a transparent supernatant for glucose measurement. The glucose oxidase peroxidase kit was utilized to quantify the glucose content in each aliquot. The total starch hydrolysis amount (TSH, %) was calculated based on the glucose content ($G_t$) produced at each hydrolysis time and the total starch content (m) in the sample. The formula for this calculation was as follows,

$$TSH = \frac{G_t \times 0.9}{m} \times 100$$

Rapidly RDS, SDS, and RS can be determined using the following formulas,

$$RDS(\%) = (G_{20} - FG) \times 0.9/TS$$

$$SDS(\%) = (G_{120} - G_{20}) \times 0.9/TS$$

$$RS(\%) = [TS - (RDS + SDS)]/TS$$

G20 represents the glucose released after 20 min of enzymatic hydrolysis. G120 represents the glucose released after 120 min of enzymatic hydrolysis by amylase. FG denotes the free glucose content present before the enzymatic hydrolysis process. TS stands for the total starch content in the sample.

### 2.7. Calculation of hydrolysis index and eGI

The *in vitro* digestion of simulated chestnut flour was conducted according to the statistical method described by Coñi et al. (Goñi, Garcia-Alonso, & Saura-Calixto, 1997). To determine the starch hydrolysis curve of chestnut flour, the following kinetic equation was utilized, $C = C_\infty (1\text{-}e^{-kt})$.

In this equation, C represents the percentage of starch hydrolyzed at a given time, $C_\infty$ represents the maximum possible hydrolysis level, k is the rate constant, and t represents the time of hydrolysis. By fitting this model to the experimental data, the values of $C_\infty$ and k can be obtained, providing insights into the kinetics of starch hydrolysis during the simulated digestion of chestnut flour.

The area under the curve of hydrolysis (AUC) is a metric used to quantify extent of enzymatic hydrolysis over time. It represents the integrated response of the hydrolysis process and can be calculated using the following formula,

$$AUC = C_\infty (t_f - t_0) - (C_\infty/k)\left[1 - e^{\left[-k(t_f - t_0)\right]}\right]$$

Where $t_f$ is the final reaction time (180 min), $t_0$ is the initial reaction time (0 min). The eGI was calculated using the formula,

$$eGI = 39.71 + 0.549HI$$

The determination of water holding capacity (WHC) follows the method described by Liu et al. with minor modifications (Liu et al., 2020). Briefly, 0.1 g of the sample (designated as $m_1$) was weighed and placed into a 10 mL centrifuge tube. The combined weight of the centrifuge tube and the sample was then recorded as $m_2$. Subsequently, 5 mL of distilled water was added, and the mixture was thoroughly mixed. The tube was then centrifuged at 4000 r/min. After centrifugation, the supernatant was discarded, and the weight of the centrifuge tube and the sediment was measured as $m_3$. The WHC was calculated using the formula,

$$WHC\ (g/g) = (m_3 - m_2)/m_1 \times 100\%$$

For the determination of swelling degree (S) and solubility($S_d$), the method of Keeratiburana et al. (Keeratiburana, Hansen, Soontaranon, Tongta, & Blennow, 2020) was adopted with minor modifications. Initially, 0.10 g of chestnut flour (designated as $m_0$) was accurately weighed and transferred into a centrifuge tube containing 5 mL distilled water. The mixture was then heated at 60 °C for 30 min while being continuously mixed. Following this, the mixture was centrifuged at 4000 r/min for 15 min. The supernatant was then transferred to an evaporating dish and dried to a constant weight at 105 °C. The mass of the dried supernatant represents the dissolved starch (designated as m). The solubility (S) and swelling degree ($S_d$) were calculated as follows,

$$S\ (\%) = m/m_0 \times 100$$

$$Sd\ (ml/g) = V/m_0$$

Where V represents the volume of the supernatant.

### 2.8. Statistical analysis

Origin 2021 software (OriginLab, Northampton, MA, USA) was employed for mapping and visualizing the data results. Additionally, Excel 2021 v2212 (Microsoft, Raymond, WA, USA) and SPSS v22.0 (IBM, Amonk, NY, USA) were utilized for the processing and statistical analysis of all collected data. Specifically, Duncan's test was applied to compare the data between different treatment groups, with a significance level of $p < 0.05$. To ensure reproducibility and reliability, three biological replicates were measured for each group of processed data.

**Table 2**
Effects of different color protection liquid formulations on the color of chestnut flour during processing.

| Group | Vc content | | | Citric acid content | | | EDTA-$Na_2$ contents | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| L* | 85.83 ± 0.06 cd | 85.70 ± 0.50 cd | 87.13 ± 0.06ab | 87.53 ± 0.15a | 85.63 ± 0.15d | 87.13 ± 0.06ab | 85.90 ± 0.35 cd | 86.87 ± 0.23b | 86.10 ± 0.10c |
| a* | −2.17 ± 0.32ab | −2.37 ± 0.32b | −3.23 ± 0.25 cd | −3.43 ± 0.21d | −2.30 ± 0.17b | −2.23 ± 0.06ab | −1.87 ± 0.06a | −3.00 ± 0.17c | −2.10 ± 0.17ab |
| b* | 32.40 ± 0.00ab | 32.30 ± 0.40ab | 32.33 ± 0.06ab | 32.13 ± 0.21b | 32.63 ± 0.06a | 30.47 ± 0.06d | 31.77 ± 0.06c | 32.37 ± 0.12ab | 31.67 ± 0.26c |
| ΔE | 7.93 ± 0.28 cd | 8.06 ± 0.17c | 9.11 ± 0.23ab | 9.33 ± 0.28a | 8.13 ± 0.17c | 7.61 ± 0.05de | 7.40 ± 0.10e | 8.86 ± 0.16b | 7.60 ± 0.26de |

Note: different lowercase letters in the same line indicate significant differences ($p < 0.05$).

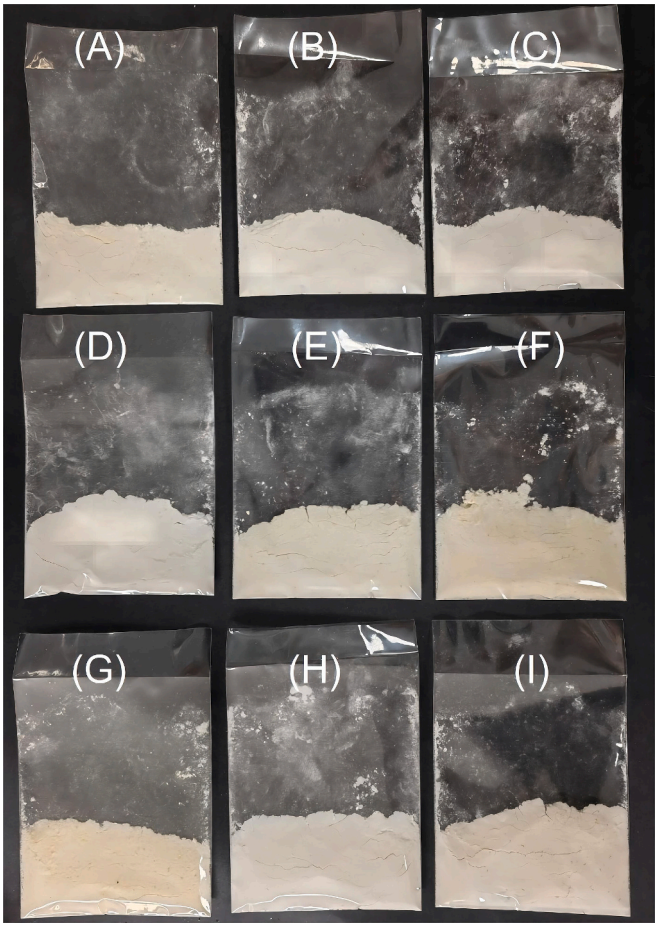



**Fig. 1.** The appearance and texture of chestnut flour treated with different color-preserving reagents. The reagents used were (A) 0.1 % Vc, (B) 0.5 % Vc, (C) 0.9 % Vc, (D) 0.9 % Vc + 0.1 % citric acid, (E) 0.9 % Vc + 0.5 % citric acid, (F) 0.9 % Vc + 0.9 % citric acid, (G) 0.9 % Vc + 0.1 % citric acid +0.01 % EDTA-$Na_2$, (H) 0.9 % Vc + 0.1 % citric acid +0.03 % EDTA-$Na_2$, (I) 0.9 % Vc + 0.1 % citric acid +0.05 % EDTA-$Na_2$.

## 3. Results and discussion

### 3.1. Effect of color protection liquid formula on browning of chestnut flour

Table 2 presented the alterations in the pink lustre of chestnuts under various treatment methods. A experiment with three distinct factors and three levels was conducted to assess the impact of each factor on the pink lustre of chestnuts through quantitative analysis. The L* value represented the brightness of the material, while a* and b* indicated the red/green and yellow/blue hues, respectively. The L* values under different treatment methods have little difference, which may be due to the small difference in light absorption and refraction due to the similar particle size. The recorded values of L*, a*, and b* collectively suggested that the pink hue of chestnuts tends to exhibit a yellowish-green tint with a bright appearance (Fig. 1). The effectiveness of color protection is assessed through the browning degree, which is quantitatively measured by the $\Delta E$ value. The greater the $\Delta E$ value, the lighter the browning degree, and *vice versa* (Singh et al., 2011). During heating, the free carbonyl group of reducing sugar reacts with the amino group of protein components to form Schiff base (intermediate compound). Schiff base will then undergo various rearrangement and polymerization reactions, and form brown color known as melanoidin (Wani, Hamid, Hamdani, Gani, & Ashwar, 2017). Furthermore, the oxidation of phenolic compounds during the processing of chestnut flour served as a significant contributor to its browning phenomenon (Fan, Guo, Li, & Jiang, 2023). Through the comparison of different factors, it was concluded that the best parameters for color protection were 0.9 % Vc, 0.1 % citric acid, and 0.03 % EDTA-$Na_2$, and there were significant differences between each factor group ($p < 0.05$). Consequently, the optimal combination of Vc, citric acid, and EDTA-$Na_2$ for browning control was achieved at a mass ratio of 9:1:0.3, respectively.

**Table 3**
Starch content of natural chestnut flour and enzymatically hydrolyzed chestnut flour.

| Sample | Starch content (g·100 $g^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| Native chestnut flour | WR-HAD 56.21 ± 0.82b | SC-HAD 51.89 ± 0.34d | FC-HAD 50.57 ± 1.02e | WR-FD 63.21 ± 0.44a | SC-FD 53.67 ± 0.34c | 45.16 ± 0.14f |
| Enzymatic hydrolysis of chestnut flour | FH-ER1 53.21 ± 0.89a | FH-ER2 44.25 ± 1.58b | FH-ER3 42.75 ± 2.43b | WF-ER1 53.94 ± 1.30a | WF-ER2 43.75 ± 0.87b | WF-ER3 41.48 ± 1.60b |

### 3.2. Starch content analysis of enzymatic hydrolysis chestnut flour

Table 3 presents a comparison of starch content between native chestnut flour and enzymatic hydrolysis chestnut flour. Among the various treatment methods employed for whole raw chestnut flour, vacuum freeze-drying treatment yielded the highest starch content, reaching 63.22 g/100 g. Conversely, for fully cooked chestnut flour, vacuum freeze-drying treatment resulted in the lowest starch content, amounting to 45.16 g/100 g. With regards to hot drying treatment, the starch content slightly lower compared to freeze drying treatment. This observation can be attributed to the gelation reaction of starch during high-temperature treatment of chestnuts, leading to a decrease in its content (Fig. 2A). Hot drying treatment, another commonly employed method, produced a starch content slightly lower than freeze drying. This observation aligns with previous studies indicating that high-temperature treatments can promote gelatinization of starch, resulting

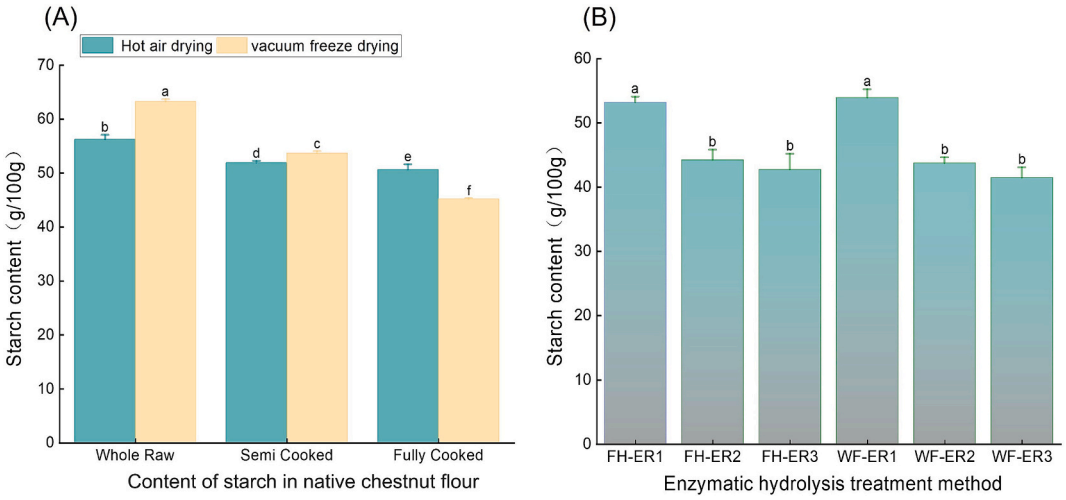



**Fig. 2.** Starch content of native chestnut flour and enzymatic hydrolyzed chestnut flour.

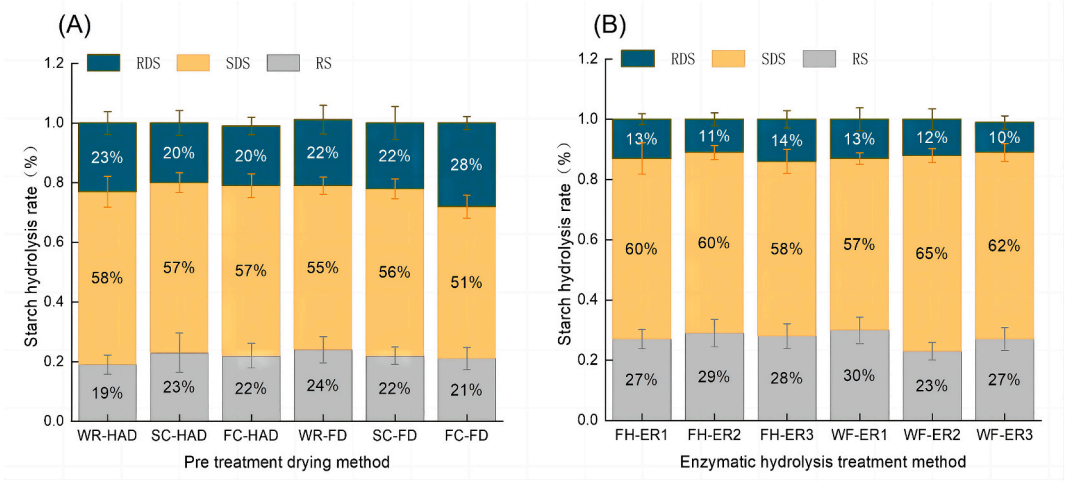



**Fig. 3.** Content of RDS, SDS, and RS in native chestnut flour and enzymatic hydrolyzed chestnut flour.

in changes to its physical and chemical properties (Ai & Jane, 2015). Gelatinization leads to the swelling and partial breakdown of starch granules, which may explain the decrease in starch content observed in our study. In addition, chestnut flour in vacuum and low temperature environment is more conducive to preservation (Zhonghe Wang, Wang, Zhang, & Huang, 2020). Different pretreatment methods affect the starch content. The whole raw pretreatment method has the highest starch content. With the reduction of cooked starch content, it may be because the integrity of chestnut cells is damaged during cooking, resulting in the loss of starch. It may also be due to the decomposition reaction caused by the damage of starch particles during pre-processing, thus reducing its content (Jiranuntakul, Puttanlek, Rungsardthong, Puncha-arnon, & Uttapap, 2012).

In the enzymatic hydrolysis of chestnut flour, the starch content decreased in the enzymatically treated samples compared to the untreated samples, and the degree of reduction differed among various enzymatic treatments. Notably, the treatment with single enzyme pullulanase resulted in the least reduction, indicating that pullulanase had a limited enzymatic hydrolysis effect. By contrast, the other two combinations of enzymes treatment caused the most significant decrease in starch content (a reduction of 34.38 %), although there was no statistically significant difference ($P > 0.5$) among them. This suggests that the enzymatic hydrolysis effects of the two combinations of enzymes were comparable (Fig. 2B). The enzymatic hydrolysis of chestnut flour is an effective method to modify its starch content, with varying degrees of reduction observed depending on the enzymatic treatment used. The high content of amylopectin in chestnut starch provides an abundant source of energy (Punia Bangar, Ashogbon, Singh, Chaudhary, & Whiteside, 2022). However, the high level of phenolic compounds interferes with the efficiency of pullulanase degradation of starch by potentially interacting with the enzyme and reducing its catalytic activity. As an exoamylase, glucoamylase plays a crucial role in starch degradation, specifically in the hydrolysis of terminal α-1,4-glycosidic bonds of starch and related polysaccharides, releasing glucose molecules (Choton et al., 2024). This characteristic is especially important in the hydrolysis of external chain segments of amylopectin (Giuberti, Rocchetti, & Lucini, 2020). Compared with the interaction between phenolic compounds and enzymes, there was less information about the interaction between phenolic compounds and starch.

### 3.3. *Digestion characteristics of enzymatic hydrolysis chestnut flour*

Fig. 3 presents a comparative analysis of the starch fractions in both native and enzymatically hydrolyzed chestnut flour. Specifically, the native flour exhibits a range of RDS content from 20 % to 28 %, SDS content from 51 % to 58 %, and RS content from 19 % to 24 % (Fig. 2A). Upon enzymatic hydrolysis, significant changes in the starch composition are observed. The RDS content decreases to 11 % to 14 %, while SDS

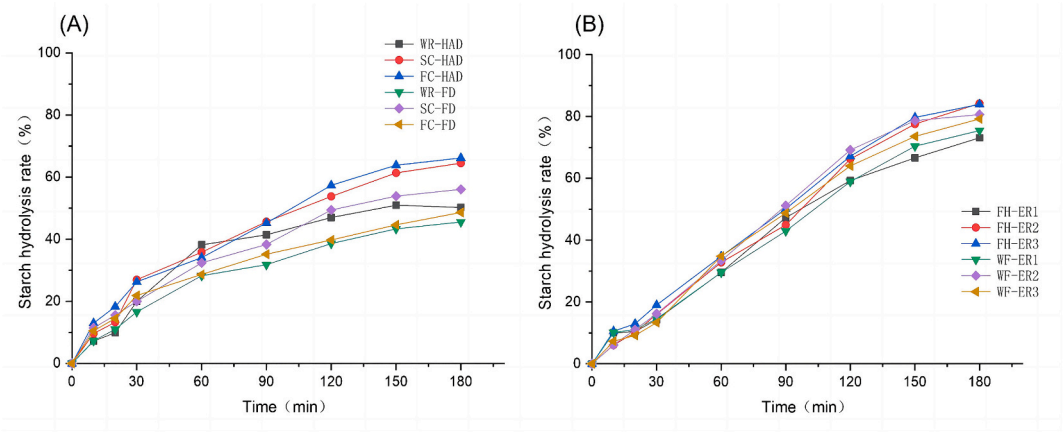



**Fig. 4.** Starch hydrolysis curves of native and enzymatic hydrolyzed chestnut flour.

and RS fractions increase to 57 % to 65 % and 23 % to 30 %, respectively (Fig. 2B). Following enzymatic hydrolysis, a notable decrease was observed in the RDS content of chestnut flour, accompanied by a concurrent increase in SDS and RS fractions. This enzymatic treatment resulted in a reduced *in vitro* digestion rate of chestnut starch. This phenomenon can be attributed to the enzymatic degradation of larger starch molecules into smaller ones, leading to a decrease in starch content and an increase in small molecular substances. The content of SDS and RS in Corn starch increased significantly after treatment with branching enzyme (Li et al., 2014). The research results of Lu et al. indicate that the combination of pullulanase and ultrasound treatment can effectively improve the yield of SDS and RS of pea starch (Lu, Belanger, Donner, & Liu, 2018). Similarly, Huang et al. reported that debranching and heat-moisture treatments significantly increased the content of SDS and RS in sweet potato starch. This result is attributed to the linear starch chain produced by debranching, which has better mobility during heat-moisture treatments, and can be rearranged effectively, thus reducing the accessibility of starch to digestive enzymes (Huang, Zhou, Jin, Xu, & Chen, 2016).

The effectiveness of single enzyme treatment was inferior to that of complex enzyme treatment due to the distinct functions of each enzyme. Pullulanase primarily targets the α-1,6 glycosidic bond, α-amylase targets the internal α-1,4 glycosidic bond of starch, and glucoamylase can hydrolyze both α-1,4 glycosidic and α-1,6 glycosidic bonds, albeit at a slower rate for the latter. Consequently, compound enzyme treatment exhibits a superior enzymatic hydrolysis effect compared to single enzyme treatment. Research has demonstrated that the linear amylose obtained following starch debranching treatment exhibits enhanced mobility, facilitating the formation of starch crystals and a single-spiral starch-lauric acid complex, as well as a double-spiral starch structure. These structure minimize the accessibility of digestive enzymes to starch (Zhang, Huang, Luo, & Fu, 2012).

### 3.4. Hydrolysis digestibility of chestnut flour

Fig. 4 depicts the starch hydrolysis rate of native and enzymatically treated starches. Notably, the starch hydrolysis rate following enzymatic treatment was significantly elevated compared to native starch, ranging from 78.37 %–89.20 %. This enhancement can be attributed to enzymatic treatment altering the molecular structure of chestnut starch, thereby exposing binding sites between the chestnut and amylase to a certain extent. Consequently, this modification significantly impacted its sensitivity. In the hydrolysis of native chestnut flour, the hydrolysis rate of chestnut flour starch was 45.54 %–66.17 %. The hydrolysis rate of the whole raw flour was significantly lower than that of the semi mature and fully mature chestnut flour, which may be due to the rupture of cell tissue caused by high temperature damage, resulting in different degrees of pre gelatinization of starch particles (H. Wang et al., 2022).

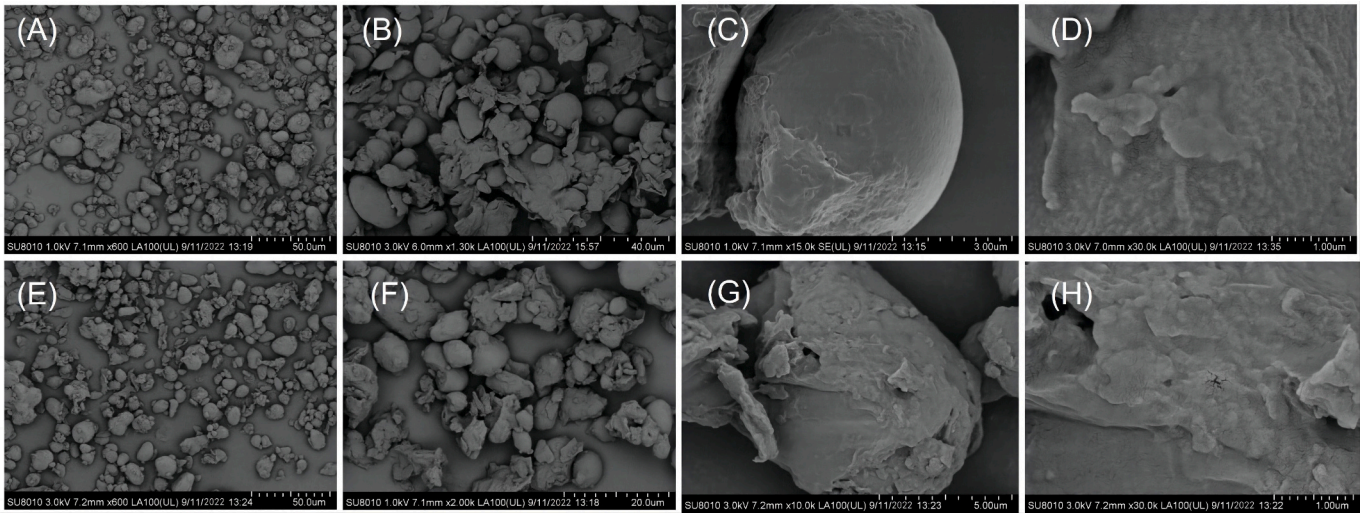



**Fig. 5.** Scanning electron microscopy analysis of the morphology of chestnut starch after enzymatic hydrolysis. (A)-(D) is an electron microscope picture of the native starch particles, (*E*)-(H) is a scanning electron microscope picture of the enzymatically hydrolyzed starch particles. The scale in the bottom right corner of the image represents the resolution size of the picture.

**Table 4**
Index values of native chestnut flour and enzymatic hydrolyzed chestnut flour.

| Different treatments | $C_\infty$ | $k$ | AUC | HI | GI |
|---|---|---|---|---|---|
| WR-HAD | 51.97 | 0.01497 | 6117.54 | 32.11 | 57.34 |
| SC-HAD | 67.00 | 0.01154 | 6981.80 | 36.64 | 59.83 |
| FC-HAD | 70.04 | 0.01157 | 7308.73 | 38.36 | 60.77 |
| WR-FD | 47.69 | 0.01188 | 5042.91 | 26.47 | 54.24 |
| SC-FD | 58.03 | 0.01237 | 6260.38 | 32,86 | 57.75 |
| FC-FD | 44.72 | 0.01665 | 4682.84 | 24.58 | 53.20 |
| FH-ER1 | 130.07 | 0.00476 | 7687.19 | 40.35 | 61.86 |
| FH-ER2 | 252.26 | 0.00235 | 8381.43 | 43.99 | 63.86 |
| FH-ER3 | 157.51 | 0.00443 | 8814.23 | 46.26 | 65.14 |
| WF-ER1 | 174.36 | 0.00328 | 7681.64 | 40.32 | 61.85 |
| WF-ER2 | 155.62 | 0.00441 | 8678.02 | 45.55 | 64.72 |
| WF-ER3 | 158.61 | 0.00404 | 8262.56 | 43.37 | 63.52 |

The enzymatic modification of starches resulted in a reduction of paste viscosity, the demonstration of distinct rheological properties, an enhancement of starch elastic behavior, and an improvement in digestibility(Singla et al., 2020). These studies support the notion that enzymatic modification can effectively alter the rheological properties and digestibility of starches.

### 3.5. Scanning electron microscopic analysis of enzymatic hydrolyzed flour

The microstructure of natural and modified chestnut flour was observed by scanning electron microscope (SEM). Most native chestnut starch is circular in shape and has a relatively smooth surface (Fig. 5A-D). After enzymatic hydrolysis, most of the starch particles are irregularly shaped, with significant changes in particle size. The surface becomes rough, and some of the surface structures of the particles are uneven and have pores or holes, indicating that the starch particles are damaged and rearranged (Fig. 5E-H).

The formation principle of RS can be summarized as the water absorption and expansion of the original starch in the gelatinization state, resulting in the destruction of its structure. After releasing straight chain branched molecules, they undergo low-temperature retrogradation and rearrangement, and the molecular chains approach each other to form a double helix structure, which is then combined through hydrogen bonds to form a stable crystal structure (Wang et al., 2023). The microstructure of the two types of starch particles in the figure is relatively consistent. The debranched starch sample exhibits an irregular shape and a dense particle structure. Zeng et al. reported that the dense starch structure has high resistance to enzymatic hydrolysis(F. Zeng, Zhu, Chen, Gao, & Yu, 2016).

### 3.6. Hydrolysis index and eGI of enzymatic hydrolysis chestnut flour

Table 4 presents the pertinent indicators pertaining to the hydrolysis index and blood glucose generation index of both native and enzymatic chestnut flour. These indices encompass parameters such as $C_\infty$, $k$, AUC, Hydrolysis index (HI), and eGI, all of which are essential in assessing the physiological and nutritional properties of the flour. The eGI value of native chestnut flour varies between 53.20 and 60.77, with certain samples exhibiting relatively low eGI values. In contrast, the enzymatic hydrolyzed chestnut flour exhibits a eGI range of 61.85 to 65.14, which is a categorized as a medium eGI value. In addition, the GI value of the freeze-dried samples was significantly lower than that of the hot-dried samples. Another study revealed that the eGI of hot- dried and freeze-dried chestnut flour was higher, whereas the eGI of vacuum-dried, roasted, and phenolic-pretreated hot-air dried chestnut flour was lower than that of naturally dried chestnut flour (Yang, Zhang, Wu, & Ouyang, 2022). In recent years, products with low eGI values have become very popular, mainly because low eGI foods can slow down the body's digestion and absorption of carbohydrates and starch, and maintain blood sugar balance (Hao et al., 2018). Native chestnut flour has a low eGI and can slowly increase blood sugar. Although it can reduce cardiovascular and other diseases, the hydrolysis rate of native chestnut flour is low, and it stays in the gastrointestinal tract for a long time, which can burden the digestive system. Therefore, foods with low eGI values are not suitable for all populations, particularly elderly individuals and young children with poor digestive capabilities.

### 3.7. Hydration properties of enzymatic hydrolysis chestnut flour

Water holding capacity is an important index of physicochemical properties of edible flour. The greater the water holding capacity, the stronger the water absorption capacity. Fig. 6 showed the water holding capacity of native chestnut flour and enzymatic hydrolysis chestnut flour. The water holding capacity of native chestnut flour was 0.9–3.1, and that of enzymatic hydrolysis chestnut flour was 5.3–8.6. The water holding capacity of raw chestnut flour increased from 0.9 to 3.1 under hot air drying and from 1.0 to 2.0 under vacuum freeze drying, indicating that cooking treatment improved the water holding capacity of raw chestnut flour (Fig. 6A). After enzymolysis, the structure of chestnut flour was affected by enzyme, indicating the formation of irregular pores. The highest water holding capacity of chestnut flour was 8.6 after hot air drying and enzymatic hydrolysis by compound enzyme/

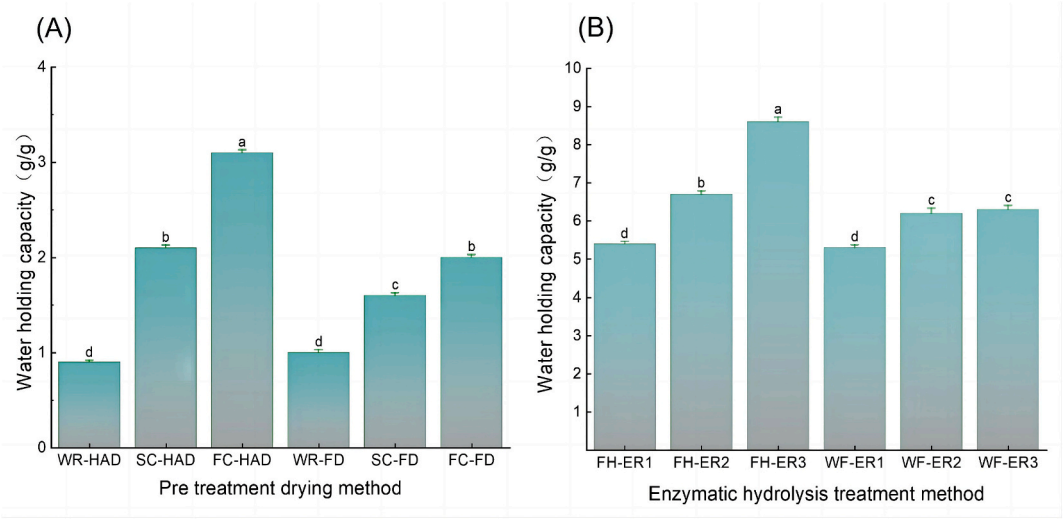



**Fig. 6.** Determination of water holding capacity of native chestnut flour and enzymatic hydrolyzed chestnut flour.

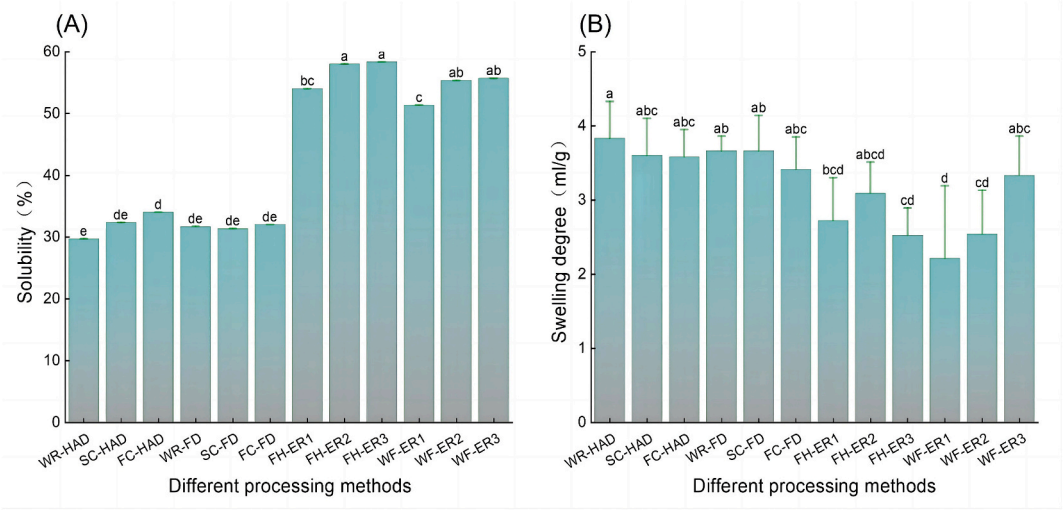



**Fig. 7.** Solubility and swelling degree of chestnut flour after different pretreatment and enzymatic hydrolysis.

pullulanase. The lowest water holding capacity was the chestnut flour which was hydrolyzed by single pullulanase after vacuum freeze-drying, and the water holding capacity was 5.3 (Fig. 6B). The water holding capacity of enzymolysis treatment was much higher than that of native chestnut, indicating that enzymolysis treatment significantly improved the water holding capacity of chestnut samples. Enzymatic hydrolysis treatment causes partial degradation of the internal structure of starch and becomes loose, exposing hydrophilic groups such as carboxyl and hydroxyl groups on the surface, thereby increasing the water holding capacity of starch (Chen, 2022). Strong water holding capacity improved food quality by reducing dehydration shrinkage, which was usually necessary for functional foods. Enzymatic hydrolysis treatment lead to the exposure of hydrophilic groups, change the spatial structure and the ratio between crystalline and amorphous regions, and makes it easier for water molecules to enter the fiber structure(Ma et al., 2022).

### *3.8. Analysis of solubility and swelling*

The solubility is related to the degree of dissolution of amylose in the process of starch swelling, and the swelling degree reflects the strength of starch's ability to absorb water and retain water(Cai et al., 2015). Fig. 7 showed the swelling degree and solubility of native chestnut flour and enzymatic hydrolysis chestnut flour. The solubility of chestnut increased from 29.67 % – 34.00 % to 51.33 % –58.33 % after enzymolysis, which may be due to the increase of amylose after enzymatic hydrolysis (Fig. 7A). The swelling degree of chestnut flour decreased from 3.41 to 3.83 mL/g to 2.21–3.33 mL/g after enzymolysis (Fig. 7B). The swelling ability of chestnut flour decreased after enzymolysis. Studies have shown that the solubility is usually related to amylose, while the swelling capacity is related to amylopectin. The enzymatic hydrolysis treatment led to the breakage of starch granules. The first component released from the granules was amylose, which improved the solubility. The result of particle breakage is a decrease in viscosity and an increase in fluidity(Punia Bangar et al., 2022). At the same time, it has also been reported that amylose leaching during dry heat treatment will increase the solubility (Gou et al., 2019). In addition, after the amylase hydrolysis of chestnut, the content of free starch chain in the solution increases, and the hydrogen bonding between the starch reduces the exposure of polar groups in the starch, the chance of combining starch with water molecules, and the swelling power of starch (Xie, 2022).

## 4. Conclusion

The current study aimed to simulated the *in vitro* digestion of both native and enzymatically hydrolyzed chestnut flour, comparing their digestion characteristics and evaluating the effects of enzymatic hydrolysis and color protection on chestnut flour's microstructure, hydrolysis index, blood glucose index, solubility, swelling degree, and digestibility. Key findings revealed that the optimal composition for the color protection solution of chestnut flour was a mass ratio of 9:1:0.3 of Vc, citric acid, and EDTA-$Na_2$. Both heat treatment and compound enzyme hydrolysis treatment led to a reduction in starch content. Following enzymatic hydrolysis, the content of RDS decreased, while the content of SDS and RS increased. The hydrolysis rate of enzymolyzed chestnut flour was higher compared to native chestnut flour, indicating that enzymatic treatment enhances digestibility. eGI predictions revealed that native chestnut flour had a eGI value ranging from 53.20 to 60.77. Enzymatic hydrolysis chestnut flour exhibited a eGI value between 61.85 and 65.14, categorized as medium eGI. Enzymatic hydrolysis of chestnut flour exhibits moderate eGI values, ease of digestion, and favorable color attributes. These features render it an ideal substitute product for individuals with abnormal sugar metabolism, offering both strong satiety and digestibility.

## CRediT authorship contribution statement

**Wenxin Guo:** Software, Formal analysis. **Liyang Yang:** Methodology, Formal analysis. **Xinyu Shi:** Data curation. **Xin Cong:** Funding acquisition. **Shuiyuan Cheng:** Project administration. **Linling Li:** Supervision, Conceptualization. **Hua Cheng:** Writing – review & editing, Writing – original draft, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## References


Ashogbon, A. O. (2021). Dual modification of various starches: Synthesis, properties and applications. *Food Chemistry, 342*, Article 128325. https://doi.org/10.1016/j.foodchem.2020.128325

Cai, J., Man, J., Huang, J., Liu, Q., Wei, W., & Wei, C. (2015). Relationship between structure and functional properties of normal rice starches with different amylose contents. *Carbohydrate Polymers, 125*, 35–44. https://doi.org/10.1016/j.carbpol.2015.02.067

Chen, Y. (2022). Effects of enzymolysis and fermentation treatment on quality properties and microstructure of CCMRP. *Shenyang Normal University, Shenyang.*. https://doi.org/10.27328/d.cnki.gshsc.2022.000327

Cheng, H., Zha, S., Luo, Y., Li, L., Wang, S., Wu, S., Cheng, S., & Li, L. (2022). JAZ1-3 and MYC2-1 synergistically regulate the transformation from completely mixed flower buds to female flower buds in *Castanea mollisima*. *International Journal of Molecular Sciences, 23*(12), 6452. https://doi.org/10.3390/ijms23126452

Cheng, L., Wu, W., Li, J., Lin, X., Wen, J., Peng, J., Yu, Y., Zhu, J., & Xiao, G. (2023). Effect of heat transfer medium and rate on freezing characteristics, color, and cell structure of chestnut kernels. *Foods, 12*(7), 1409. https://doi.org/10.3390/foods12071409

Choton, S., Bandral, J. D., Singh, J., Bhat, A., Sood, M., Gupta, N., … Kaur, D. (2024). Enzymatic modification of starch: A review. *Saudi J Med Pharm Sci, 10*(1), 1–8. https://doi.org/10.36348/sjmps.2024.v10i01.001

Englyst, H. N., Kingman, S. M., & Cummings, J. H. (1992). Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition, 46*(2), S33–S50.

Eyinla, T. E., Sanusi, R. A., & Maziya-Dixon, B. (2021). Effect of processing and variety on starch digestibility and glycemic index of popular foods made from cassava (Manihot esculenta). *Food Chemistry, 356*, Article 129664. https://doi.org/10.1016/j.foodchem.2021.129664

Fan, X., Guo, S., Li, Y., & Jiang, X. (2023). Study on correlation between browning degree of chstnut fruit and total phenols and flavonoids. *Journal of Nanjing Forestry University (Natural Sciences Edition), 47*(6), 159–166. https://doi.org/10.12302/j.issn.1000-2006.202111009

Giuberti, G., Rocchetti, G., & Lucini, L. (2020). Interactions between phenolic compounds, amylolytic enzymes and starch: An updated overview. *Current Opinion in Food Science, 31*, 102–113. https://doi.org/10.1016/j.cofs.2020.04.003

Goñi, I., Garcia-Alonso, A., & Saura-Calixto, F. (1997). A starch hydrolysis procedure to estimate glycemic index. *Nutrition Research, 17*(3), 427–437. https://doi.org/10.1016/S0271-5317(97)00010-9

Gou, M., Wu, H., Saleh, A. S. M., Jing, L., Liu, Y., Zhao, K., … Li, W. (2019). Effects of repeated and continuous dry heat treatments on properties of sweet potato starch. *International Journal of Biological Macromolecules, 129*, 869–877. https://doi.org/10.1016/j.ijbiomac.2019.01.225

Hao, H., Li, Q., Bao, W., Wu, Y., & Ouyang, J. (2018). Relationship between physicochemical characteristics and *in vitro* digestibility of chestnut (*Castanea mollissima*) starch. *Food Hydrocolloids, 84*, 193–199. https://doi.org/10.1016/j.foodhyd.2018.05.031

Huang, T., Zhou, D., Jin, Z., Xu, X., & Chen, H. (2016). Effect of repeated heat-moisture treatments on digestibility, physicochemical and structural properties of sweet potato starch. *Food Hydrocolloids, 54*, 202–210. https://doi.org/10.1016/j.foodhyd.2015.10.002

Jiranuntakul, W., Puttanlek, C., Rungsardthong, V., Puncha-arnon, S., & Uttapap, D. (2012). Amylopectin structure of heat–moisture treated starches. *Starch, 64*(6), 470–480. https://doi.org/10.1002/star.201100160

Keeratiburana, T., Hansen, A. R., Soontaranon, S., Tongta, S., & Blennow, A. (2020). Porous rice starch produced by combined ultrasound-assisted ice recrystallization and enzymatic hydrolysis. *International Journal of Biological Macromolecules, 145*, 100–107. https://doi.org/10.1016/j.ijbiomac.2019.12.144

Lee, E., Song, E., Nam, Y., Nam, T., Kim, H., Lee, B., Seo, M., & Seo, D. (2020). Effects of enzymatically modified chestnut starch on the gut microbiome, microbial metabolome, and transcriptome of diet-induced obese mice. *International Journal of Biological Macromolecules, 145*, 235–243. https://doi.org/10.1016/j.ijbiomac.2019.12.169

Li, R., Sharma, A. K., Zhu, J., Zheng, B., Xiao, G., & Chen, L. (2022). Nutritional biology of chestnuts: A perspective review. *Food Chemistry, 395*, Article 133575. https://doi.org/10.1016/j.foodchem.2022.133575

Li, X., Miao, M., Jiang, H., Xue, J., Jiang, B., Zhang, T., Gao, Y., & Jia, Y. (2014). Partial branching enzyme treatment increases the low glycaemic property and α-1, 6 branching ratio of maize starch. *Food Chemistry, 164*, 502–509. https://doi.org/10.1016/j.foodchem.2014.05.074

Liu, S., Yu, J., Zou, J., Yang, Y., Cui, L., & Chang, X. (2020). Effects of different drying and milling methods on the physicochemical properties and phenolic content of hawthorn fruit powders. *Journal of Food Processing and Preservation, 44*(6), Article e14460. https://doi.org/10.1111/jfpp.14460

Lu, Z., Belanger, N., Donner, E., & Liu, Q. (2018). Debranching of pea starch using pullulanase and ultrasonication synergistically to enhance slowly digestible and resistant starch. *Food Chemistry, 268*, 533–541. https://doi.org/10.1016/j.foodchem.2018.06.115

Ma, Q., Ma, Z., Wang, W., Mu, J., Liu, Y., Wang, J., Stipkovits, L., Hui, X., Wu, G., & Sun, J. (2022). The effects of enzymatic modification on the functional ingredient - dietary fiber extracted from potato residue. *LWT, 153*, Article 112511. https://doi.org/10.1016/j.lwt.2021.112511

Park, E. Y., Ma, J.-G., Kim, J., Lee, D. H., Kim, S. Y., Kwon, D.-J., & Kim, J.-Y. (2018). Effect of dual modification of HMT and crosslinking on physicochemical properties and digestibility of waxy maize starch. *Food Hydrocolloids, 75*, 33–40. https://doi.org/10.1016/j.foodhyd.2017.09.017

Punia Bangar, S., Ashogbon, A. O., Singh, A., Chaudhary, V., & Whiteside, W. S. (2022). Enzymatic modification of starch: A green approach for starch applications. *Carbohydrate Polymers, 287*, Article 119265. https://doi.org/10.1016/j.carbpol.2022.119265

Singh, G. D., Riar, C. S., Saini, C., Bawa, A. S., Sogi, D. S., & Saxena, D. C. (2011). Indian water chestnut flour- method optimization for preparation, its physicochemical, morphological, pasting properties and its potential in cookies preparation. *LWT-Food Science and Technology, 44*(3), 665–672. https://doi.org/10.1016/j.lwt.2010.09.015

Singla, D., Singh, A., Dhull, S. B., Kumar, P., Malik, T., & Kumar, P. (2020). Taro starch: Isolation, morphology, modification and novel applications concern - a review. *International Journal of Biological Macromolecules, 163*, 1283–1290. https://doi.org/10.1016/j.ijbiomac.2020.07.093

Trinidad, T. P., Mallillin, A. C., Sagum, R. S., & Encabo, R. R. (2010). Glycemic index of commonly consumed carbohydrate foods in the Philippines. *Journal of Functional Foods, 2*(4), 271–274. https://doi.org/10.1016/j.jff.2010.10.002

Wang, H., Wang, P., Duan, Y., Huo, J., Wang, K., & Xiao, Z. (2022). Effect of cooking treatment on cell structure and digestive characteristics of whole potato flour. *Science and Technology of Food Industry, 43*(7), 87–93. https://doi.org/10.13386/j.issn1002-0306.2021070142

Wang, L., Shi, D., Chen, J., Dong, H., & Chen, L. (2023). Effects of Chinese chestnut powder on starch digestion, texture properties, and staling characteristics of bread. *Grain & Oil Science and Technology, 6*(2), 82–90. https://doi.org/10.1016/j.gaost.2023.01.001

Wang, Z., Wang, J., Zhang, J., & Huang, R. (2020). Effect of processing on content of free amino acid, carbohydrate and *in vitro* digestibility of starch in *Euryales ferox*. *Food and Fermentation Industries, 46*(5), 166–173. https://doi.org/10.13995/j.cnki.11-1802/ts.022772

Wang, Z., Wang, S., Xu, Q., Kong, Q., Li, F., Lu, L., Xu, Y., & Wei, Y. (2023). Synthesis and functions of resistant starch. *Advances in Nutrition, 6*(1), 1131–1144. https://doi.org/10.1016/j.advnut.2023.06.001

Wani, I. A., Hamid, H., Hamdani, A. M., Gani, A., & Ashwar, B. A. (2017). Physico-chemical, rheological and antioxidant properties of sweet chestnut (*Castanea sativa* mill.) as affected by pan and microwave roasting. *Journal of Advanced Research, 8*(4), 399–405. https://doi.org/10.1016/j.jare.2017.05.005

Xie, Y. (2022). *Synergistic enzymolysis interaction of polyphenols regulates retrogradation and digestibility of chestnut starch*. Henan Zhenzhou: Unpublished Food Science and Engineering, Henan University of Technology. https://doi.org/10.27791/d.cnki.ghegy.2022.000103

Yang, Z., Wu, Y., & Ouyang, J. (2023). Effect of cooking method and enzymatic treatment on the in vitro digestibility of cooked and instant chestnut flour. *Plant Foods for Human Nutrition, 78*(1), 166–172. https://doi.org/10.1007/s11130-022-01035-5

Yang, Z., Zhang, Y., Wu, Y., & Ouyang, J. (2022). The role of drying methods in determining the *in vitro* digestibility of starch in whole chestnut flour. *LWT, 153*, Article 112583. https://doi.org/10.1016/j.lwt.2021.112583

Zeng, F., Zhu, S., Chen, F., Gao, Q., & Yu, S. (2016). Effect of different drying methods on the structure and digestibility of short chain amylose crystals. *Food Hydrocolloids, 52*, 721–731. https://doi.org/10.1016/j.foodhyd.2015.08.012

Zeng, X., Wang, M., Chen, L., & Zheng, B. (2024). Impact of using whole chestnut flour as a substitute for cake flour on digestion, functional and storage properties of chiffon cake: A potential application study. *Food Chemistry, 432*, Article 137016. https://doi.org/10.1016/j.foodchem.2023.137016

Zhang, B., Huang, Q., Luo, F., & Fu, X. (2012). Structural characterizations and digestibility of debranched high-amylose maize starch complexed with lauric acid. *Food Hydrocolloids, 28*(1), 174–181. https://doi.org/10.1016/j.foodhyd.2011.12.020

Zhu, C., Liu, L., Wang, M., Zhao, Y., Chen, Y., Lu, X., & Geng, G. (2019). A new late-ripening Chinese chestnut cultivar, Jianding-youli. *HortScience, 54*(4), 758–760. https://doi.org/10.21273/hortsci13743-18