Effect of ultrasonic pretreatment on eliminating cyanogenic glycosides and hydrogen cyanide in cassava

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ABSTRACT

Traditional soaking method takes days to remove cassava cyanide. Ten minutes of ultrasonic pretreatment (UPT) was found to be a new effective method to eliminate both cyanogenic glycosides and hydrogen cyanide in cassava. Here, the parameters of UPT were optimized and the underlying mechanisms were investigated. 40.36% and 24.95% of hydrogen cyanide and cyanogenic glycosides in cassava juice were eliminated under 10 min of UPT (45°C, 81 W). UPT before boiling enhanced the total cyanide elimination to 41.94%. The degradation patterns of hydrogen cyanide and cyanogenic glycosides were different. Ultrasound directly eliminated hydrogen cyanide and indirectly degraded cyanogenic glycosides through promoting enzymatic hydrolysis. The β-glucosidase activity was increased by 17.99% induced by ultrasound. This was supported by the movement of hydrophobic residual and the rearrangement of the secondary structure of the molecule as found in fluorescence, CD, FTIR, DSC and TG analysis. This study revealed that UPT acted as a fast and simple technical way in improving cassava safety.

1. Introduction

Food poisoning induced by endogenous toxins is a global food safety issue. Cyanogenic glycoside is a group of common natural toxin in food [1]. Cyanogenic glycoside can be degraded into cyanide, which is highly toxic and harms the reproductive and neural system [2]. Cassava root is consumed as staple food by one billion people in Africa tropical and subtropical areas [3]. It contains over 90% starch at dry basis. It is easy to cultivate because it resists to drought and needs little fertilizer. Cassava is a promising crop in food security, but the high content of cyanogenic glycoside obstructs its spread. Linamarin and lotaustralin are the major cyanogenic glycosides in cassava [4]. A series of poisoning incidents caused by the consumption of cassava has been widely reported and symptoms such as syncope, vomiting, and even death were seen [2,3]. Previous work of our research group has assessed the dietary exposure risk of consuming cassava in China [4]. There is a potential acute risk due to the high cyanogenic glycoside residues in cassava juice [4]. Proper food processing is required to reduce the health concerns of cyanide-containing foods.

The decontamination of cyanides in foods like almonds, apricots, cassava, flaxseeds and bamboo shoots has been widely researched in the past years [3,5–7]. The Codex Alimentarius Commission has announced corresponding removal measures to protect public health [8]. Cyanide in cassava is mainly presented in the form of cyanogenic glycosides and hydrogen cyanide (free HCN/CN⁻) [9]. The ratio of free cyanide is around 2–5% and the glycosylated cyanide was up to 95–98% [10]. Fig S1 shows the hydrolysis of cyanogenic glycosides of cassava. Firstly, the glycosidic bond can be hydrolyzed by β-glucosidase (EC 3.2.1.21) during
processing [9]. The intermediate degradation product is unstable cyanohydrin and then it spontaneously dissociates into the final highly toxic hydrogen cyanide when pH > 4 [3]. The hydrolysis of cyanogenic glycosides by β-glucosidase is the rate-limiting step to remove cassava cyanide [3].

Traditional strategies such as boiling, steaming and baking have a certain degree of removal effect [3]. However, β-glucosidase is easily inactivated during thermal processing and cassava starch is also gelatinized after the process, which limiting their application as a pretreatment in cassava juice production. Soaking is also a typical pretreatment way employed in cassava processing. Fig S2 shows the processing of cassava juice involved with soaking pretreatment. In Africa, cassava is usually soaked in water for several days before fermentation [11,12]. In China, it is customary to soak for 1–4 h in the industry. According to scientific researches, soaking is not an efficient way for cyanide elimination [12]. The potential dietary exposure risk indicated that the effectiveness and efficiency of processing need improving [4]. It has been reported the application of ultrasonic-assisted processing in the food industry, including extraction of phytochemicals and degradation of the organic and inorganic pollutants [13,14]. Ultrasound has been widely studied to eliminate food contaminants such as pesticides, mycotoxins and heavy metals [14]. Investigations on sono-enzymatic degradation effect on food contaminants and mechanisms from mechanical energy generated by ultrasound have been discussed [15,16]. Due to the effect of ultrasonic cavitation, which promotes biomass transference and modifies enzymatic structure, the enzymatic reaction efficiency was enhanced [17,18]. The investigation of ultrasonically modifying the β-glucosidase activity during soybeans soaking, almonds debittering and ougan juice debittering were reported [19–21]. Zhang et al. [20] reported that ultrasound could accelerate the translocation of amygdalin from apricot kernels into debitterizing water. These efforts provided an innovative green technique to eliminate contaminants by food processing. However, whether ultrasound can reduce cyanogenic glycosides or hydrogen cyanide in cassava and their mechanisms have not been well studied.

Ultrasonic pretreatment (UPT) with mild intensity and temperature would possibly activate the enzymatic hydrolysis of cyanogenic glycosides, and accelerate the removal of its degraded products. Hence, to investigate the hypothesis, this article was to (1) investigate the condition of short-time and low-intensity UPT on the effect of cyanide elimination, including the cyanogenic glycosides and hydrogen cyanide, (2) explore the β-glucosidase activity during ultrasound-assisted enzymatic hydrolysis against cyanogenic glycosides, (3) verify the structural and physicochemical changes of the β-glucosidase after ultrasound to explore the mechanism of sono-enzymatic degradation. UPT is expected to be used in the control of cyanide in cassava as well as many other cyanide-containing food products.

2. Materials and methods

2.1. Chemicals and reagents

Standards of linamarin (98%) and lotaustralin (97%) were obtained from TRC (Toronto, Canada). Cyanogen in water (GBWE) 080115) at 50 μg/mL was produced by the Chinese Academy of Metrology. Acetonitrile and methanol (HPLC grade) were from Sigma-Aldrich (Steinheim, Germany). Chloramine-T, isonicotinic acid, barbituric acid and β-glucosidase were obtained from Sigma-Aldrich (Chicago, China). All other reagents including sodium hydroxide, monopotassium phosphate, disodium hydrogen phosphate were of analytical grade purchased from Sinopharm Co., Ltd. (Shanghai, China). Ultrapure water used in the experiments was prepared by Milli-Q purification system (Millipore, Bedford, USA).

2.2. Cassava treatment

2.2.1. Preparation of cassava

Fresh cassava (variety of SC9) collected from Hainan province was cleaned with water. Then it was peeled manually and cut into one cubic centimeter. After mixed evenly, cassava was placed in a sealing sample bag and stored at −20°C for further use.

2.2.2. Soaking

Traditional soaking treatment was operated at room temperature in clean water (200 g: 300 mL) for 0 to 24 h based on a previous report [11]. After soaking for a while, cassava was taken out from the water, drained, and ready for cyanide detection.

2.2.3. Preparation of cassava juice

500 g of cassava was squeezed with 1000 mL of prechilled clean water and filtered through a cotton cloth. The cassava juice collected was immediately sealed at a glass bottle and stored in the refrigerator.

2.2.4. Ultrasonic pretreatment

50 mL of juice was put in a jacked beaker and treated with ultrasound with a diameter of 4.5 mm probe of 22 kHz ultrasound irradiation (JY92–IIDN, Ningbo Scientz Biotechnology Co., Ningbo, China). The generated maximum power was 900 W. Water-bath was collected with the jacked beaker to control the temperature. A temperature sensor was collected with the ultrasonic generator to monitor the temperature. UPT temperatures (25–75°C), power (0–225 W) and duration (0–30 min) were conducted. The untreated group was conducted at the same temperature and same time but without ultrasound. Cyanide in cassava including cyanogenic glycosides and hydrogen cyanide were then detected.

2.3. Cyanide determination

Cyanogenic glycosides were extracted and analyzed by UHPLC-QqQ-MS/MS (Agilent 1290 Infinity UHPLC, Agilent Technologies, USA) according to the method developed previously [4,22]. 1 g of cassava was extracted with 5 mL of 80% methanol aqueous solution and then shaken for 15 min. After centrifugation at 4200 g for 5 min, the supernatant was collected and filtered through a 0.22 μm membrane filter. The concentration of linamarin and lotaustralin was quantified according to the peak area of their characteristic ions. Cyanogenic glycosides were calculated as the sum of linamarin and lotaustralin.

Hydrogen cyanide analysis was performed with the isonicotinic acid-barbituric acid method according to Nagashima et al. [23]. Firstly, the isonicotinic acid-barbituric acid solution (20 g/L) was prepared by following: 6 g of NaOH was dissolved in water, 10 g of isonicotinic acid and 5 g of barbituric acid were added and heated to dissolve, and the solution was cooled and added with water into 500 mL. This isonicotinic acid-barbituric acid solution was stored at room temperature for further use. 1 mL of cassava juice was added with 1 mL of 0.1 mol/L NaOH. Then, 3 mL of 1 mol/L KH₂PO₄ solution and 200 μL of 10 g/L chloramine T were added to the mixture and derivatized for 3 min. 5 mL of 20 g/L isonicotinic acid-barbituric acid was added into the mixture to react for 15 min at room temperature. The absorbance at 600 nm wavelength was detected. The concentration of hydrogen cyanide was calculated with a cyanide ion standard curve which was obtained under the same condition. Total cyanide was calculated as the sum of cyanogenic glycosides and hydrogen cyanide.

2.4. Extraction of β-glucosidase from cassava

The preparation of β-glucosidase from cassava was performed according to Nambisan et al. [24]. After finishing with sonication (45°C, 81 W, 10 min) or boiling (20 min), cassava was homogenized with a one-fold volume of 100 mmol/L phosphate buffer (pH 6.0), vortexed and centrifuged at 10,000 g for 15 min. The β-glucosidase extract was
precipitated by 60% ammonium sulfate and centrifuged at 10,000 g for 15 min. The precipitation was dissolved in 10 mmol/L phosphate buffer (pH 6.0) and dialyzed with Amicon Ultra-50 tubes (Millipore, Billerica, MA, USA) with a molecular mass cut-off of 30 kDa. Then the β-glucosidase was ready for activity determination.

2.5. Determination of β-glucosidase activity

The activity of β-glucosidase against linamarin was performed according to Nagashima et al. [23]. 500 µL of the β-glucosidase solution was mixed with 500 µL of 10 mmol/L linamarin solution, and then made up to 10 mL with 10 mmol/L phosphate buffer (pH 6.0). After reacting for 15 min at 30℃, the cyanide ion produced was immediately determined with the onisonicotinic acid-barbituric acid method as described in Section 2.3. One unit (U) of β-glucosidase activity was defined as 1 µmol cyanide per hour generated at 30℃ in the buffer solution (pH 6.0).

2.6. Effect of UPT on cyanogenic glycosides and β-glucosidase activity

To investigate ultrasound-assisted enzymatic hydrolysis against cyanogenic glycosides, Ultrasonic treatment on substrate and enzyme was performed. 10 mmol/L linamarin and lotatustrin solution were prepared and treated with sonication (45℃, 81 W, 10 min). 1 mg/mL of β-glucosidase solution was prepared and the effect of UPT condition on β-glucosidase activity was performed as described in Section 2.2.4. 20 mL β-glucosidase solution was treated with sonication at different temperatures (25-75℃), power (0-225 W) and duration (0-30 min). After sonication, the activity of β-glucosidase standard against linamarin was detected as described in Section 2.5.

2.7. Intrinsic fluorescence spectroscopy analysis

After treated with different ultrasonic power (0-225 W), intrinsic fluorescence spectra of the β-glucosidase solution (1 mg/mL) were measured with a Cary Eclipse fluorescence spectrophotometer (Varian Inc., Palo Alto, USA). The fluorescence intensity was performed at the fixed excitation wavelength of 280 nm and the emission wavelength of 300 to 500 nm.

2.8. Circular dichroism analysis (CD)

The CD spectra of β-glucosidase solution (1 mg/mL) with sonication (45℃, 81 W, 10 min) were collected with a circular dichroism spectrometer (JASCO J1500, Tokyo, Japan). Scanning was performed at a range of 190 to 260 nm at 100 nm/min with 1 nm of bandwidth in three replicates. Data of the secondary structure was analyzed using online DICHROWEB.

2.9. Fourier transform infrared (FTIR) analysis

The FTIR spectrum was collected on a spectrometer (Thermo Nicoleti550, WI, USA). The β-glucosidase solution (1 mg/mL) was treated with sonication (45℃, 81 W, 10 min) and freeze-dried. The prepared dried powder was fully mixed with potassium bromide (1:100) and compressed for detection. Scanning was performed at range of 400 to 4000 cm⁻¹ at the resolution of 4.0 cm⁻¹ with total scans of 32. The characteristic spectra then was analyzed with OMNIC 8.2 software (Thermo Fisher Scientific, USA).

2.10. Thermal properties analysis

The thermal properties were performed on β-glucosidase (1 mg/mL) of freeze-dried. The differential scanning calorimetry (DSC) and thermogravimetric analysis (TG) were obtained with a Thermal analyzer (Mettler Toledo International Inc., Switzerland). The sample was placed in platinum pans and heated from 30 to 180℃ at a rate of 10 °C/min with nitrogen (20 mL/min).

2.11. Statistical analysis

Results were described as mean ± standard deviation of three independent measurements. Statistical analyses of variance (ANOVA, P < 0.05) were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Figures were obtained with originLab Corp., MA, USA.

3. Results and discussion

3.1. Effect of UPT condition on cassava cyanide elimination

To establish an effective method for cyanide removal, UPT was investigated here. Ultrasonic conditions including temperature, power, and duration were optimized for cyanide elimination. Overall, a higher elimination rate was found with the aid of the UPT for both hydrogen cyanide and cyanogenic glycosides under all conditions studied (Fig. 1). The optimal condition of UPT on total cyanide in cassava juice was suggested at 45℃, 81 W for 10 min, under which 24.95% of the total cyanide was eliminated. For hydrogen cyanide, the elimination was faster than formation resulted from cyanogenic glycosides hydrolysis.

The hydrogen cyanide and cyanogenic glycosides were reduced from 1.96 mg/kg to 1.17 mg/kg and from 179.8 mg/kg to 134.9 mg/kg with the aid of sonication at the optimal condition. The corresponding removal rate of hydrogen cyanide and cyanogenic glycosides was 40.36% and 24.95%, respectively. As the temperature rose within the range of 25-75℃, the effectiveness of UPT on hydrogen cyanide increased. It might be caused by the thermal instability of hydrogen cyanide [9]. The elimination of cyanogenic glycosides did not increase along with the temperature increase, indicating the cyanogenic glycosides were mainly degraded through the endogenous enzymes but not heat. As the ultrasonic power increased from 36 to 225 W, the elimination of hydrogen cyanide was increased, but 81 W was preferred for cyanogenic glycosides. Many reports showed that ultrasonic treatment under low power intensity adding to modifying the reactions between enzymes and substrates [25]. Within the 30 min of sonication, the elimination of cyanogenic glycosides and hydrogen cyanide was increased with a longer treatment duration. Ultrasound speeded up the change of food chemical composition by damaging the cell wall and membrane and promoting the heat and mass transference [13].

It has not been noticed previously that the effect of UPT condition on hydrogen cyanide and cyanogenic glycosides removal was different. Early researches found that ultrasonic treatment could increase the decomposition efficiency of free cyanide [7,26]. The reason was possibly related to the ultrasonic oxidation by the radicals generation, and the accelerated evaporation by the transient high temperature [9,26]. Here, the elimination of cyanogenic glycosides was found to be closely related to enzymatic hydrolysis.

3.2. Effect of soaking treatment on cyanide elimination

Soaking was an ancient processing method used to remove food contaminants or to produce fermented food. In cassava, flaxseed, bamboo shoots, almonds and soybean, soaking had been widely applied to remove cyanide [3,5,19,27,28]. The experimental results showed that short-term soaking did not have good effects (Table 1). 14.38% of hydrogen cyanide was removed after soaking for 4 h, but only negligible cyanogenic glycosides were removed (2.56%). Similar results were also found in the earlier studies. Around 20% of hydrogen cyanide was removed after immersing cassava for 4 h [12]. 40% of total cyanide was retained after soaking grated cassava for 80 h of fermentation [29]. This induced the challenge of wastewater and it usually consumes a long...
time. For improving the effect of soaking, measurements such as delaying the soaking time to 3–5 days, or cooperating with the extra process were used [3,12,30]. As reported by Zhang et al. [20], 61.58% of the D-amygdalin in apricot kernels was eliminated by 2 h of 55°C water, and this effect was enhanced to 85.43% with the aid of ultrasound bath of 2 h, 55°C, 300 W. Here, 24.95% of the total cyanide was removed with the assisting of UPT for only 10 min. Thus, ultrasound was evidenced to be time-saving in cassava cyanide elimination compared with soaking.

### 3.3. Effect of β-glucosidase activity on cyanide elimination

To further investigate the UPT effect and its mechanism, different treatments including UPT, boiling, and combination of them were conducted. Based on the optimal condition we had obtained in Section 3.2, UPT on cassava was conducted at 45°C, 81 W for 10 min. The hydrogen cyanide, cyanogenic glycosides, and endogenous β-glucosidase were monitored between those different treatments. Both thermal and enzymatic degradation was observed from Table 2. Here, 31.54% of the total cyanide was decreased after boiling for 20 min. Previous study found the cyanogenic glycosides in cassava were decreased by 39% after boiling for 30 min [4]. What’s more, 41.94% of the total cyanide was decreased significantly when UPT was added before boiling (P < 0.05). However, it was interesting that ultrasound was no longer effective after boiling—the total cyanide remained the same (68.03%) statistically as that of boiling alone (68.46%). The activity of endogenous β-glucosidase in cassava was increased from 5.54 to 6.21 U when UPT was performed. It indicated that the endogenous β-glucosidase was activated by ultrasound. After boiling, the enzyme was inactivated [3], so that the elimination of cyanogenic glycosides was no longer increased when UPT was applied after boiling. Zhang et al. [20] found that endogenous β-glucosidase in apricot kernels contributed 10.37% of the D-amygdalin removal. The different effects of ultrasound on cyanide elimination showed consistency with the result shown in Fig. 1.A.
On the other side, hydrogen cyanide showed a different result. The hydrogen cyanide was reduced to 69.54% when UPT was added before boiling. And hydrogen cyanide was reduced to 70.76% when UPT was added after boiling. The order of ultrasound did not influence the hydrogen cyanide elimination.

Recently, studies found that in organic or inorganic contaminants such as mycotoxins or pesticides, whose molecules were possibly involved with mechanochemical bond breakdown and rearrangement during sonication although the mechanism was not so clear yet [14,25,31,32]. Results showed that the increased degradation of the cyanogenic glycosides might be caused by changes in enzymatic hydrolysis promoted by ultrasound. Therefore, the influence of UPT in enzymatic hydrolysis needed further investigation.

3.4. Effect of UPT on enzymatic hydrolysis

As showed in Fig. 2.A, the concentration of cyanogenic glycosides substrate was not changed after ultrasound. It can be concluded that cyanogenic glycosides were not directly degraded by ultrasound. To further convince that the glycosylated cyanide was degraded by enzymatic hydrolysis, the enzymatic activity was checked. The effects of temperature, power, and duration on the enzymatic reaction of β-glucosidase against linamarin were explored. Results showed that enzymatic activity was increased after UPT (Fig. 2.B-D). The maximum activity was increased by 17.99% under the optimal condition (45°C, 81 W, 10 min), which was consistent with the results of Fig. 1. The effect of ultrasound-assisted activation on β-glucosidase was found to be enzyme-specific, and also closely related to the ultrasonic condition [33]. The highest activity of endogenous β-glucosidase in soybean occurred at 45°C ultrasound as described in the previous study [19]. Reports on cyanogenic β-glucosidase activity had revealed the optimal temperature ranged from 35 to 55°C in apricot and black plum seed [34–36]. Generally, low-intensity and short-time ultrasound could activate the enzyme, but high-intensity and long-time ultrasound may cause enzyme inactivation [17,18,25,37].

3.5. Intrinsic fluorescence spectroscopy analysis

To verify the structural and characteristic changes of the β-glucosidase during ultrasound-assisted enzymatic hydrolysis, analysis including intrinsic fluorescence spectroscopy, Fourier transform infrared (FTIR), circular dichroism (CD), the differential scanning calorimetry (DSC) and thermogravimetric analysis (TG) were following conducted [17]. The endogenous fluorescence spectra of β-glucosidase with ultrasonic pretreatment at the fixed excitation wavelength of 280 nm were shown in Fig. 3. Compared with untreated sample, the peak of the strongest emission wavelength showed a weak red-shifted (350–360 nm), and the fluorescence intensity was decreased after sonication. The endogenous fluorescence of the enzyme protein was excited by the aromatic amino acid residues in the enzyme molecule. Tyrosine and tryptophan produced fluorescence intensity at 280 nm, and the fluorescence changes of tryptophan residues were very sensitive to the changes of the microenvironment of the protein. Therefore, the red-shifted and the decreased intensity indicated the movement of the chromophores. 

![Fig. 2. Effect of ultrasonic pretreatment (UPT) on cyanogenic glycosides and β-glucosidase activity. (A) Cyanogenic glycosides; (B) Temperature; (C) Power; (D) Duration.](image)

![Fig. 3. Effect of ultrasonic pretreatment (UPT) on fluorescence spectra.](image)
hydrophobic residues of the β-glucosidase molecule. Li et al. [38] found that the fluorescence intensity of peroxidase was increased with the increasing ultrasonic power (0–90 W). Hou et al. [37] reported that ultrasonic treatment resulted in a decrease in the fluorescence emission value of chitinase. Both the increased and decrease intrinsic fluorescence intensity of water-soluble protein when treated with the increasing ultrasonic power (0–650 W) were found by Tang et al. [39]. There was a relative difference of the changes of the intrinsic fluorescence intensity after sonication. The reason was possibly related to the different structure of the protein, in which the chromophoric groups exposed in a different way [39]. But all those results reflected the increase of enzymatic activity was induced by the stretch of the protein different structure of the protein, in which the chromophoric groups fluoresce intensity after sonication. The reason was possibly related to the microenvironment of the enzymatic structure and aided the interaction of the enzyme and substrate.

3.6. CD analysis

The structure of protein always changed with the activity of the enzyme, acted as strong evidence of the characteristic change. The CD spectra reflected the corresponding liquid protein secondary structure information at far-ultraviolet wavelength (Fig. 4A). Generally, the positive peak at around 192 nm and the negative valley at the range of 200–220 nm referred to the α-helix structure; the positive peak at 190 nm referred to the β-sheet structure; the random coil structure had a weak positive peak at around 220 nm [28]. The secondary structure constitution of β-glucosidase with ultrasonic pretreatment was shown in Fig. 4B. Compared with untreated β-glucosidase, the content of α-helix and β-sheet was reduced from 37.0% to 34.2%, and from 19.3% to 19.2%, while the content of random coil and β-turn was increased from 31.7% to 34.3%, and from 11.1% to 12.3% after sonication. This indicated the disorder of the secondary structure was increased and it was consistent with the effect of the acoustic cavitation [13]. Influence of acoustic cavitation on the enzyme unfolding and active site exposing by ultrasound was put forward [13].

3.7. FTIR analysis

The characteristic peak at 1648 and 1654 cm⁻¹ from FTIR spectra was identified as amide I band of the untreated and sonication samples, respectively. The increased wavenumber of amide I band indicated the hydrogen bond was reduced after sonication [39]. The reason was likely involved in the disruption of hydrogen network by ultrasonic shearing force [38]. Since amide I band (1700 to 1600 cm⁻¹) was a sensitive wavenumber for the changes in protein secondary structure, it was usually used to characterize the protein secondary structure. The FTIR spectrum extracted from 1700 to 1600 cm⁻¹ was displayed in Fig. 4C-D. According to the previous reports, the secondary structure by FTIR was identified as following: β-sheet at 1625 cm⁻¹ and 1626 cm⁻¹, random coil at 1642 cm⁻¹ and 1643 cm⁻¹, α-helix at 1658 cm⁻¹ and 1660 cm⁻¹, β-turn 1675 cm⁻¹ 1676 cm⁻¹. After sonication, the content of α-helix and β-sheet was reduced from 33.1% to 30.3%, from 22.3% to 20.0%, and the random coil and β-turn were increased from 28.3% to 32.6%, from 16.3% to 17.1%. As reported by Hou et al. [37], activation of chitinase induced by ultrasound was accompanied by the reduced α-helix and the increased random coil when treated by ultrasound. When the ultrasonic power was increasing, the content of α-helix was increased firstly and then decreased, and the content of random coil was decreased firstly and then increased. Similar variation of water-soluble protein during the increasing ultrasonic power treatment was reported by Tang et al. [39]. Generally speaking, the activation of the enzyme was likely related to the exposure of active sites, which was induced by rearrangement of the secondary structure [38].

3.8. DSC analysis

The thermal properties of the β-glucosidase were found to be influenced by the ultrasound. As shown in Fig. 5A-B, the value of ΔH was decreased from 59.83 J/g to 24.85 J/g after sonication, indicating the decrease of the orderliness of the enzymatic structure [39]. The thermal denaturation temperature was 67.52°C and showed a weak increase with ultrasound of 63.01°C. Change of the T½ reflected the thermal stability was possibly affected by the ultrasound. Fan et al. [28] reported that after 31 min of ultrasound, the ΔH value of β-glucosidase was

Fig. 4. Effect of ultrasonic pretreatment (UPT) on secondary structure constitution.
decreased from 43.44 J/g to 10.13 J/g, and the \( T_d \) value was decreased from 72.36 °C to 71.64 °C. The change of enthalpy property was consistent with the secondary structural change obtained by CD and FTIR. The chemical mechanism of ultrasound-assisted enzymatic hydrolysis was revealed previously by lowering the enthalpy [40]. It can be further deduced that rearrangement of the secondary structure such as the increased orderliness structure, resulted in the characteristic changes of the \( \beta \)-glucosidase during ultrasound-assisted enzymatic hydrolysis.

3.9. TG analysis

The TG and DTG curves were shown in Fig. 5 C-D. Firstly, the free water was evaporated at 30-132 °C [41]. The weight loss of the \( \beta \)-glucosidase was 4.86% of untreated and 7.02% of UPT at this stage. The major weight loss was occurred at the second stage accompanying by carbonaceous material decomposition [41]. At the second stage, 24.86% of the weight the \( \beta \)-glucosidase was decreased, and 24.88% of the weight was decreased after sonication under the thermal degradation. 0.62% and 0.74% of the weight loss occurred at the last stage caused by the impurities. The major weight loss of the protein was 26.14% when calculated at dry basis, and 26.75% after sonication, which showed a weak increase with ultrasound. This finding supported the changes of enzymatic structure as had been found in fluorescence, CD, FTIR and DSC analysis.

Overall, all those results proved that the enzymatic hydrolysis was modified by change of microenvironment, including the movement of the hydrophobic residual of the molecular and the rearrangement of the secondary structure that induced by ultrasound [17].

4. Conclusions

From the previous investigation, we had learned that dietary exposure from cassava cyanogenic glycoside has a potential acute risk, which has derived from the high level of cyanogenic glycoside in cassava juice. The traditional soaking method kept the cassava in water for several hours to days. Results showed that short-term soaking did not have a good effect on cyanide elimination (only 3% of removal after 4 h), and it required a long time and produced wastewater. Ten minutes of UPT was found to be a potential way to eliminate both cyanogenic glycosides and hydrogen cyanide. Optimal UPT conditions including temperature, power and duration of hydrogen cyanide and cyanogenic glycosides removal were different. Impact on the hydrogen cyanide and cyanogenic glycosides caused by the sequence of ultrasound and boiling was found. A different elimination pattern between the hydrogen cyanide and cyanogenic glycosides was proposed. The hydrogen cyanide was sensitive to sonication and it was directly reduced by the ultrasound. Cyanogenic glycosides need to be hydrolyzed through \( \beta \)-glucosidase. The enzymatic hydrolysis against cyanogenic glycosides was improved by ultrasound due to the increase of \( \beta \)-glucosidase activity. Changes in hydrophilic residues, secondary structures, and thermal properties were found in \( \beta \)-glucosidase after sonication. For further investigation, attention to improving the thermal stability of the endogenous \( \beta \)-glucosidase, or the extra addition of \( \beta \)-glucosidase were probably meaningful for cassava cyanide elimination.

CRediT authorship contribution statement

Yongheng Zhong: Conceptualization, Methodology, Investigation, Writing – original draft. Tao Xu: Investigation, Software, Writing - review & editing. Shengyang Ji: Writing - review & editing. Xiaodan Wu: Methodology, Investigation. Tian Zhao: Writing - review & editing. Shimin Li: Methodology, Resources. Peng Zhang: Writing - review & editing, Resources. Kaimian Li: Resources. Baiyi Lu: Conceptualization, Methodology, Investigation, Funding acquisition, Supervision, Project administration.

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.

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