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Effective collection of protein-enriched cells from green tea residue: An innovative process for leaf protein production

Chen Zhang [a,b,c], Ziyang He [a,b,c], Ankun Wang [a], Feipeng Zhang [a,*]

[a] Institute of Food Science and Technology, College of Biological Science and Engineering, Fuzhou University, 350108, Fuzhou, China
[b] Fujian Center of Excellence for Food Biotechnology, 350108, Fuzhou, China
[c] Food Nutrition and Health Research Center, School of Advanced Manufacturing, Fuzhou University, 362200, Jinjiang, Fujian, China

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ABSTRACT

Green tea residue (GTR) contains a high protein content. However, the protein in GTR can't be effectively extracted using traditional methods. Thus, a novel method using ethylenediamine tetraacetic acid (EDTA), ammonium oxalate, or Celluclast® 1.5 L were used to disperse leaf tissues and to collect mesophyll cells to enrich the protein. Compared with EDTA or ammonium oxalate treatment, Celluclast® 1.5 L treatment achieved the highest amounts of mesophyll cells, about 2.7×10^6 g^{-1} of GTR. The number of collected mesophyll cells was positively and linearly correlated with the extraction rate of glucose and xylose, indicating that cellulose and hemicellulose were key components influencing cell collection. Celluclast® 1.5 L treatment enriched the protein content by 1.65 times in collected mesophyll cells to 50% protein content with a protein recovery of 88%, providing a novel scheme to obtain high-quality leaf protein for the food industry.

1. Introduction

Leaves, with protein content ranging from 16% to 29% (Tenorio et al., 2018), are one of the most abundant protein resources in the world (Hauer et al., 2021; John, 1979; Santamaría-Fernández and Lübeck, 2020), however, they are rarely utilized in the food industry (Motghare et al., 2016). As an example, green tea residue (GTR), a byproduct of tea beverage production, has a protein content from 20% to 30% (Jayasuriya et al., 1978; C. Zhang et al., 2017). However, it is usually disposed through incineration (Ozturk et al., 2019) or composting (Luo et al., 2022), causing air pollution and environmental eutrophication. Thus, exploring a sustainable technology for extracting leaf protein is essential in the food industry (Tenorio, Gieteling, de Jong, Boom and van der Goot, 2016).

GTR protein is mainly extracted using alkaline solutions or enzymatic methods, and the extracted protein is purified through thermal or acid precipitation (Iyer et al., 2021). Although the alkaline method can extract up to 95% of the protein, the alkaline pH may denature the proteins and cause unwanted side reactions (Zexin et al., 2022; C. Zhang et al., 2017). Enzymatic extraction proceeds under mild processing conditions, but yields less protein (Baker and Charlton, 2020; Vergara-Barberán et al., 2015). Thermal precipitation consumes energy and reduces protein solubility (Lamsal et al., 2006), while acid precipitation produces difficult-to-separate particles (Pirie, 1988). Thus, this study proposed and developed a novel method to obtain high-quality leaf protein for the food industry by dispersing the tissues and collecting protein-enriched mesophyll cells (Fig. 1).

The dispersion of leaf tissues determines the collection efficiency of protein-enriched cells. The mesophyll cells are mainly connected through intercellular pectin adhesion(Lionetti et al., 2015) or cross-linking of cell wall polysaccharides (Chebli et al., 2021). As a result, we speculated that mesophyll cells could be dissociated by removing intercellular pectin or degrading cell wall polysaccharides. The pectin can be removed from the mesophyll cells by chelating calcium ions with chelating agents (L. Li et al., 2021; Z. Li et al., 2023), thus disrupting the calcium cross-linking in the HG region and dissolving the pectin (Lionetti et al., 2015). In laboratory research, EDTA is a widely used chelating agent capable of forming stable complexes with various metal ions (Ravn and Meyer, 2014). Compared with EDTA, ammonium oxalate is less stable but has a strong specific effect on only calcium ions (Rosen, 2009). Additionally, ammonium oxalate is relatively cheap (Mattar et al., 2022). They are both frequently used in laboratory research on homogalacturonan (HG) pectin extraction. Apart from HG pectin in the intercellular matrix, intercellular adhesion may involve

* Corresponding author. College of Biological Science and Engineering, Fuzhou University, Address: South No.501 Guojia Keji Yuan, No.2 Xueyuan Road, Shangjie Town, Fuzhou City, 350108, China.
E-mail addresses: feipeng.zhang_fzu@qq.com, feipeng.zhang@lcb.chemie.uni-giessen.de (F. Zhang).

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rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II), cellulose, hemicellulose, lignin, and other components in the cell wall (Barnes and Anderson, 2018). The cell wall contains a complex mixture of compounds and intricate cross-links, which cannot be effectively degraded by a single enzyme (Ruangmee and Sangwichien, 2013). In this study, we selected Celluclast® 1.5 L, which has been discovered to be effective in extracting pectin from plant cell wall (Nadar et al., 2018; Sabater et al., 2018). Owing to its safety, Celluclast® 1.5 L can be utilized not only in laboratory research but also in food processing. The extraction of pectin and/or disruption of cell wall structure using the above three treatment methods has been studied, but little is known about dispersing GTR mesophyll cells and their protein enrichment effect.

In this study, protein-enriched mesophyll cells were collected using GTR as the model material. The GTR was treated with EDTA, ammonium oxalate, or Celluclast® 1.5 L, and the number of obtained cells was determined by measuring their volume and average particle size. Effects on substance release during protein enrichment was analyzed by measuring the content of released protein, and total dry weight. In addition, their degradation effect on GTR pectin and cell wall structure was assessed. The monosaccharide compositions of dissolved substances were determined to investigate the effects of specific treatments on the different pectin domains and cell wall components. Key components that affect the collection of GTR mesophyll cells were identified based on correlations between the released components and the protein content. Finally, protein enrichment effects were assessed, and the fine structure of the collected mesophyll cells was examined with a scanning electron microscope (SEM). This study provides valuable insights for future studies on GTR, protein enrichment, mesophyll cells, and protein dissolution.

2. Materials and methods

2.1. Materials

GTR was sourced from Damin Company in Fujian Province, China. The green tea leaves (*Camellia sinensis*) were extracted using hot water at 85 °C for 45 min, filtered, and then dried at 60 °C to prepare the GTR samples. The GTR was sieved using 4-mesh and 20-mesh sieves, and the leaves that passed through the 4-mesh sieve and those intercepted using the 20-mesh sieve were selected for subsequent experiments. The GTR samples contained 26% of protein, which was determined using the Kjeldahl method (Kjeldahl, 1883; Yemm and Willis, 1954).

Celluclast® 1.5 L (Cellulase from *Trichoderma reesei*) was purchased from Sigma, USA. The analytical grade EDTA, ammonium oxalate, galacturonic acid, and ferrous tartrate purchased from Sinopharm Chemical Reagent Co., Ltd, China. Bovine serum albumin was purchase from Shanghai Acmec Biochemical Co., Ltd, China. Tea polyphenols, L-(+)-rhamnose, L-(+)-arabinose, D-(+)-xylose, D-(+)-galactose, and D-(+)-glucose purchased from Shanghai Yuanye Bio-Technology Co., Ltd, China were of analytical grade.

2.2. Treatments on GTR

GTR (100 mg) was mixed with 4 mL of different solutions.

1) disodium ethylenediaminetetraacetate (EDTA, 0.1 M) at 60 °C,
2) ammonium oxalate (AO, 0.1 M) at 60 °C,
3) acetic acid-sodium acetate buffer (0.02 M, pH 4.5) containing Celluclast® 1.5 L 1.2 U at 40 °C.

The reactions involving EDTA, ammonium oxalate, and Celluclast® 1.5 L were performed using a constant temperature oscillator (WKB-100-4, Shanghai Jingxin Industrial Development Co., Ltd.) at a constant stirring of 1000 rpm and varying time intervals ranging from 0.5 to 20 h. The reactions involving EDTA and ammonium oxalate were conducted at optimized temperatures of 60 °C, and those involving Celluclast® 1.5 L at 40 °C.

2.3. Collection of GTR mesophyll cells

After the sample was centrifuged at 4800×*g* for 10 min using a centrifuge (LXJ-IIB, Anting, China), the resulting supernatant and sediment were collected. The supernatant was stored at 4 °C for subsequent use, while the sediment was subjected to a 150-μm pore size sieve. Mesophyll cell samples that passed the sieve were collected, while the retained leaf veins and epidermal cuticle were discarded. The collected mesophyll cells and other leaf tissues were subsequently freeze-dried using a freeze dryer (TNG-T98, Taicang Huamei, China) for future use.

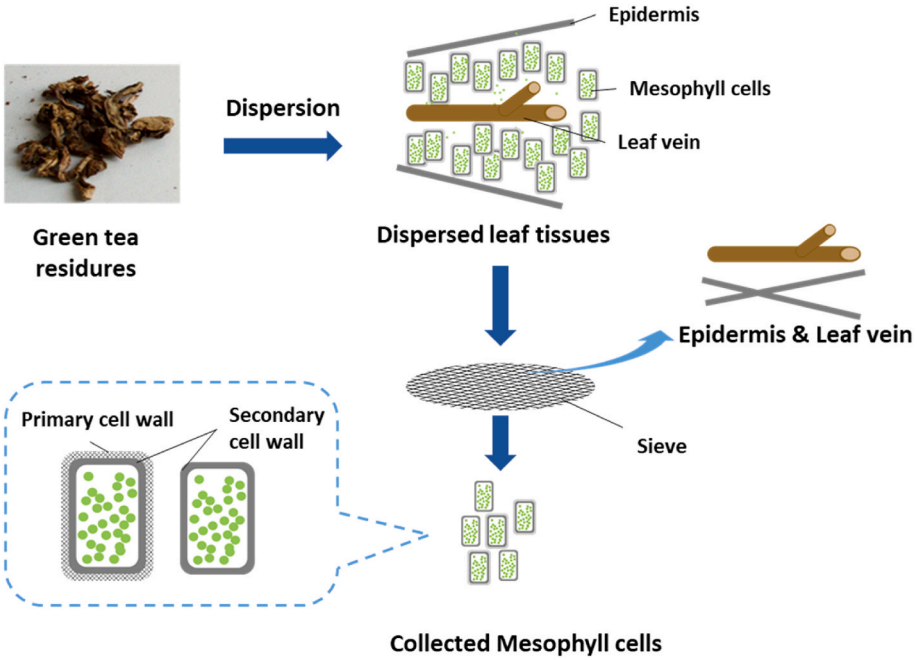

Fig. 1. The scheme for enriching tea residue proteins by collecting leaf mesophyll cells.

2.4. Determinations

2.4.1. Volume of sediment

Deionized water was added to the collected GTR mesophyll cells until a final volume of 10 mL was achieved. The mixture was then left at 4 °C for 12 h to allow the mesophyll cells to settle naturally. Then, the sediment height was recorded, and the sediment volume was calculated using 0–2.8 mL water as a reference.

2.4.2. Particle size distribution and average particle size

The particle size distribution and average diameter of collected mesophyll cells were analyzed using the Malvern laser particle size analyzer (MS 3000, Malvern Panalytical, UK). The sample exhibited a refractive index of 1.560 (D. Li et al., 2010) and an absorbance of 0.001, and water was used as the dispersant with a refractive index of 1.330. The sample concentration was 0.125% (v/v), where 0.5 mL of the suspension was dispersed in 400 mL of distilled water. The measurement was performed at a speed rate of 1200 rpm min^{-1} and a temperature of 25 °C.

2.4.3. The number of collected mesophyll cells

The number of collected mesophyll cells (N) was estimated from the sediment volume ($V_{sediment}$) and average particle size (d). The collected mesophyll cells were regarded as monosized spherical particles of equal size. The volume of a single mesophyll cell ($V_{particle}$) can be expressed as

$$V_{particle} = (4/3)\pi(d/2)^3 \quad (1)$$

The packing type of collected mesophyll cells was regarded as random loose packing with an accepted solid fraction (∅) of about 0.6 (Elmsahli and Sinka, 2020; Meng et al., 2012). The relationship between the total volume of collected mesophyll cells (V_{total}) and the volume of the sediment ($V_{sediment}$) is

$$V_{total} = \varnothing V_{sediment} \quad (2)$$

The number of collected mesophyll cells can be estimated using the following formula:

$$N = V_{total} \Big/ V_{particle} = \varnothing V_{sediment} \Big/ \left[(4/3)\pi(d/2)^3\right] \quad (3)$$

2.4.4. Microstructure observation

The collected mesophyll cells were examined using a SEM (Nova NanoSEM 230, FEI, USA). To prepare the sample for SEM imaging, 25 μL suspension of the collected mesophyll cells was dropped onto silicon wafers and allowed to dry at room temperature. Then, the dried sample was placed on a carbon binder-coated aluminum stub, sputter-coated with a thin layer of gold, and imaged using SEM at 8×kV and 5000 × magnification.

2.4.5. Dry weight

The washed sediment was collected, transferred to a 10 mL centrifuge tube, and dried in an oven at 60 °C for 48 h. The weight of the dry matter was determined using an analytical balance (TE601-L, Sartorius, Germany). The difference between the initial weight of the GTR sample and the weight of the dry matter was defined as the dry weight loss of solubles.

2.4.6. Protein content

The protein content in the extract solution was quantified using the Bradford assay with Coomassie Brilliant Blue G-250 dye. Dissolve 100 mg of Coomassie Brilliant Blue G-250 in 50 mL of 95% (v/v) ethanol, then add 100 mL of 85% (w/v) phosphoric acid. Adjust the total volume to 1000 mL with distilled water to obtain the Coomassie Brilliant Blue G-250 Protein Reagent. Aspirate 0.3 mL of the supernatant and transfer it into a centrifuge tube. Subsequently, add 1.5 mL of Coomassie Brilliant Blue G-250 Protein Reagent to the tube. Thoroughly mix the contents and allow the mixture to stand for a duration of 2 min. Following this, utilize a 1 mL cuvette to determine the absorbance of the mixture at a wavelength of 595 nm. The protein content in the sample was calculated using bovine serum albumin solution (0–0.1 mg mL^{-1}) as the reference Marion and Bradford, 1976. Protein from Celluclast® 1.5 L was deducted in the calculation.

The total nitrogen content of collected mesophyll cells was determined by a total organic carbon analyzer (TOC-L, Shimadzu, Japan),and the protein content was calculated from the total nitrogen content with a conversion factor of 6.25 (C. Zhang et al., 2016).

2.4.7. Galacturonic acid content

The galacturonic acid content in the extract was quantified using the carbazole colorimetric assay (Taylor and Buchanan-Smith, 1992). 100 μL of the diluted supernatant was pipetted into a 2-mL centrifuge tube. Subsequently, under ice-bath conditions, 1.5 mL of concentrated sulfuric acid and 50 μL of 0.1% carbazole in ethanol solution were added slowly to the tube. After thorough mixing, the reaction was allowed to proceed at 60 °C for 1 h and then terminated by immersing the tube in an ice bath. The absorbance of the sample was measured at a wavelength of 530 nm at room temperature, and the galacturonic acid content was calculated based on a standard curve ranging from 0 to 0.1 mg mL^{-1}.

2.4.8. Total sugar content

The total sugar content in the extract was determined using the anthrone sulfuric acid method (Yemm and Willis, 1954). The total sugar content in the samples was determined using a glucose solution (0–0.1 mg mL^{-1}) as the reference.

2.4.9. Polyphenol content

The polyphenol content in the extract was measured using the ferrous tartrate method (Yu et al., 2005). The polyphenols in the samples were quantified using analytical grade tea polyphenols standard (concentration range of 0–0.2 mg mL^{-1}) as the reference.

2.4.10. Neutral monosaccharide content

The types and compositions of neutral monosaccharides in the extracts were analyzed using high-performance liquid chromatography (HPLC) (UltiMate™ 3000, Thermo Fisher Scientific, USA) after 1-phenyl-3-methyl-5-pyrazolone (PMP) pre-column derivatization (Lv et al., 2008). The extract was treated with 4 mol L^{-1} of trifluoroacetic acid at 120 °C for 4 h and derivatized with PMP at 70 °C for 30 min. Reversed-phase HPLC with an Agilent C18 column (250 mm × 4.6 mm, and 5 μm of particle size) was used to separate neutral monosaccharides. The UV detector wavelength and column temperature were set at 245 nm and 25 °C, respectively, and the eluent comprised 0.1 M of phosphate buffer (pH 7) and acetonitrile in a ratio of 82:18 (v/v) at a flow rate of 1.0 mL min^{-1} and an injection volume of 10 μL. L-(+)-Rhamnose, L-(+)-arabinose, D-(+)-xylose, D-(+)-galactose (97%), and D-(+)-glucose (99.5%) were used as standard compounds. The HPLC system used was a Thermo Fisher Scientific 3000 (Thermo Fisher Scientific, USA).

2.5. Statistics

If not otherwise specified, all extracts were subjected to duplicate replicates, and two measurements were conducted for each sample (n = 4). Graphs were generated using Excel and Origin software. Data were statistically analyzed with a one-way analysis of variance using SPSS version 22.0 with a significance level of $P < 0.05$.

3. Results and discussion

3.1. Impact of three methods on GTR mesophyll cell collection efficiency

The mesophyll cells were dispersed using EDTA, ammonium oxalate,

and Celluclast® 1.5 L, and the numbers of collected mesophyll cells were calculated by their volume and mean particle size (Fig. 2).

Generally, mesophyll cell collection reached its maximum after 15 h of treatment across all three methods. However, Celluclast® 1.5 L exhibited significantly higher mesophyll cell dispersal from GTR than ammonium oxalate and EDTA (Fig. 2). The numbers of collected mesophyll cells using ammonium oxalate and EDTA treatment were approximately 3.7×10^5 and 2.8×10^5 g^{-1} GTR, respectively, which was one-twelfth or one-seventh of that achieved using Celluclast® 1.5 L. The mechanism of these methods affecting the mesophyll cell collection was further analyzed through the measurements of the composition of dissolved substances during the GTR leaf tissue dispersion.

3.2. Effects of different methods on substance dissolution

3.2.1. Effects of three methods on the composition of the dissolute components

The contents of galacturonic acid, protein, polyphenols, total sugar, and dry weight of the dissolved substance after EDTA, ammonium oxalate, and Celluclast® 1.5 L treatments were measured, and the results are presented in Fig. 3.

During the dispersion of GTR mesophyll cells using the three methods, the dry weight of the dissolved substances increased over time, reaching its peak at 10–15 h. Galacturonic acid, polyphenols, protein, and total sugar constituted the predominant components in the dissolved substance, accounting for 70–90%. Among the three methods, the contents of dissolved polyphenols and proteins showed no significant difference, while other components varied. After the sample was subjected to Celluclast® 1.5 L treatment, the dissolved galacturonic acid was 80 mg g^{-1} GTR, which was twice more than those subjected to EDTA and ammonium oxalate treatment. EDTA and ammonium oxalate only extracted a small amount of total sugars, approximately 20 and 30 mg g^{-1} GTR, respectively, which were one-tenth of the Celluclast® 1.5 L treated sample. The dissolution of galacturonic acid and total sugars represented the degradation of pectin and other polysaccharide structures in GTR. Considering the diversity and complexity of polysaccharides in GTR, the determination of monosaccharides was necessary for further analysis of the types and structures of dissolved polysaccharides.

3.2.2. Effects of ammonium oxalate and Celluclast® 1.5 L treatments on the monosaccharide composition in GTR extracts

The monosaccharide composition in GTR extracts was determined. As EDTA interfered with the derivatization process, the EDTA-treated sample was undetectable, while other results are shown in Fig. 4.

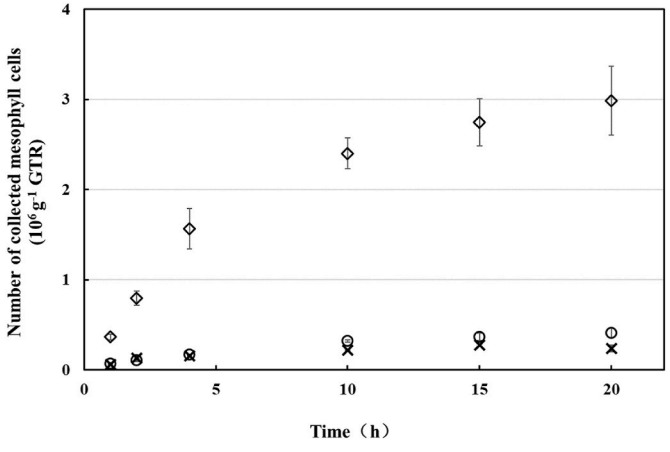

Fig. 2. Numbers of collected mesophyll cells from GTR using 0.02 M acetic acid-sodium acetate buffer (pH 4.5) containing 1.2 U Celluclast® 1.5 L at 40 °C (◇), 0.1 M ammonium oxalate at 60 °C (◯), and 0.1 M EDTA at 60 °C (×).

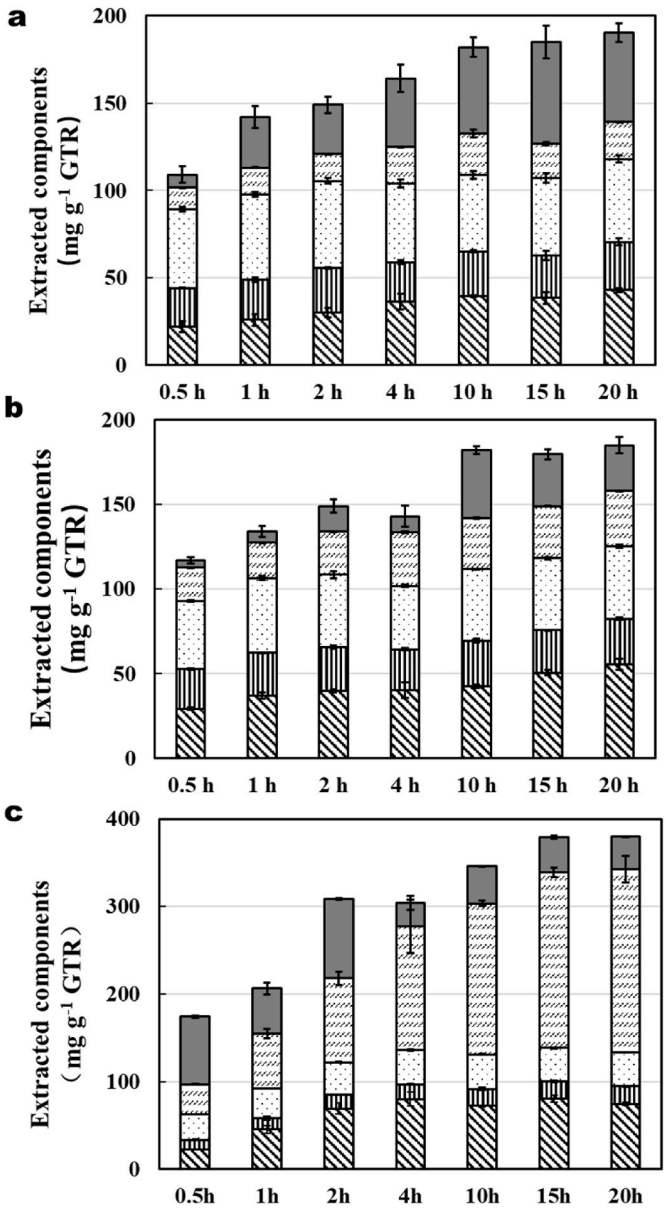

Fig. 3. Dissolved substance from GTR by using 0.1 M EDTA at 60 °C (a), 0.1 M ammonium oxalate at 60 °C (b), and 0.02 M acetic acid-sodium acetate buffer (pH 4.5) containing 1.2 U Celluclast® 1.5 L at 40 °C (c). ▧: galacturonic acid; ▥: protein; □: polyphenols; ▨: sugars; ■: other components.

Arabinose, glucose, galactose, rhamnose, and xylose were identified as the predominant monosaccharides in the analyzed samples. The monosaccharide contents in the sample after Celluclast® 1.5 L treatment was higher than that in the dissolved sample after ammonium oxalate treatment. After 20 h of Celluclast® 1.5 L treatment, the sample had the highest glucose content of 128 mg g^{-1}, which was over 10 times higher than that of ammonium oxalate treatment at the same conditions. The contents of galactose, arabinose, and rhamnose in the Celluclast® 1.5 L treatment were 30, 28, and 9 mg g^{-1}, respectively, which were 2–5 times higher than that of the ammonium oxalate treatment. The xylose content in GTR extract reached a maximum of 10 mg g^{-1} after Celluclast® 1.5 L treatment, which was hardly detected after ammonium oxalate treatment. Galactose (Artur et al., 2020) is present in HG, RG-I, and RG-II pectin, whereas rhamnose is specific to RG-I and RG-II pectin (Konstantinos, 2021). After GTR was subject to ammonium oxalate treatment, the galactose content in the dissolved GTR increased, while the rhamnose content remained unchanged, indicating that degradation

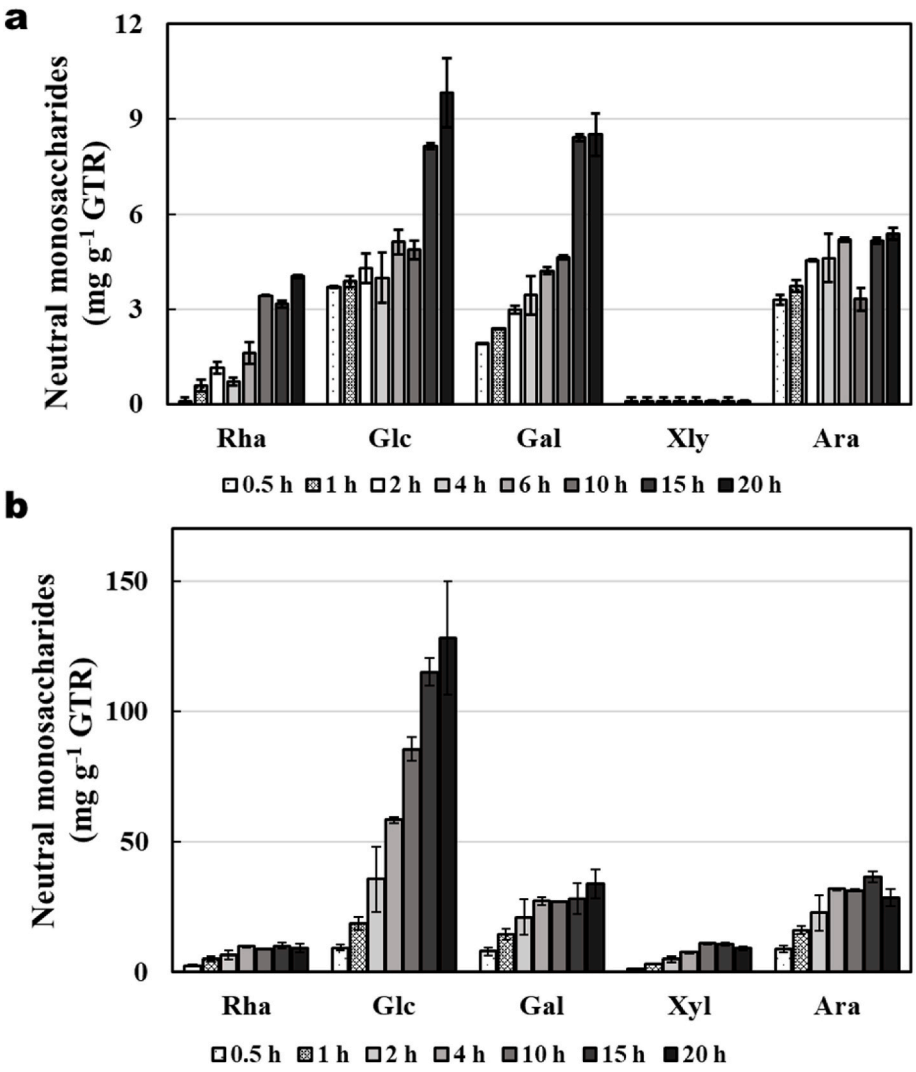

Fig. 4. Monosaccharide contents in GTR extracts treated using 0.1 M ammonium oxalate at 60 °C (a), and 0.02 M acetic acid-sodium acetate buffer (pH 4.5) containing 1.2 U Celluclast® 1.5 L at 40 °C (b).

primarily occurred in HG pectin. However, Celluclast® 1.5 L treatment significantly increased galactose and rhamnose contents, indicating its potent ability to degrade HG, RG-I, and RG-II pectin.

Glucose and xylose, as the fundamental components of cellulose and hemicellulose, constituted the primary and secondary cell walls (Arzami et al., 2022), respectively. After GTR was subjected to Celluclast® 1.5 L treatment, a notable increase in glucose content was observed in the extract, with a low xylose content. This implies that Celluclast® 1.5 L has the ability to degrade the cellulose structure of the primary cell wall, but its effectiveness in degrading the secondary cell wall is limited. Our findings show that we can obtain more mesophyll cells using the Celluclast® 1.5 L treatment than using the ammonium oxalate treatment, which is attributable to the degradation of pectin or cellulose in the primary cell wall. Thus, further analysis is needed to determine which component is more closely associated with this effect.

3.3. Key components affecting protein enrichment from GTR

The extraction rates of galacturonic acid, rhamnose, galactose, arabinose, glucose, xylose, protein, and polyphenols were plotted against the amount of collected mesophyll cells, and the results are presented in Fig. 5.

As shown in Fig. 5, three types of correlations were observed. First, the extraction rates of galacturonic acid, rhamnose, galactose, and arabinose increased with the number of collected mesophyll cells, reached their peaks, and then plateaued (Figs. 5a–d). Second, the number of collected mesophyll cells positively and linearly correlated with the extraction rate of glucose and xylose (Fig. 5e and f), with slope coefficients of 31.0 and 17.9, respectively. Third, the extraction of protein and polyphenols (Fig. 5g and h) had no effects on the mesophyll cell collection. When the extraction rate of galacturonic acid, rhamnose, galactose, and arabinose was 80%, most of the mesophyll cells remained uncollected, indicating that HG, RG-I, and RG-II pectin were not the limiting factor to the mesophyll cell collection. The extraction rate of cellulose and hemicellulose, which are the primary sources of glucose and xylose (Vanesa et al., 2023), was positively correlated with the amount of mesophyll cell collection, indicating their key roles in mesophyll cell collection. However, only cellulase slightly affected leaf tissue dispersion, indicating that the existence of pectin might hinder the degradation of cellulose or hemicellulose (N. Zhang et al., 2022).

3.4. Effects of three methods on protein enrichment of GTR

The protein content in the GTR mesophyll cells obtained using EDTA, ammonium oxalate, and Celluclast® 1.5 L was measured, and the total protein recovery rate was calculated, and the results are shown in Fig. 6a.

The collected mesophyll cells had higher protein content than GTR

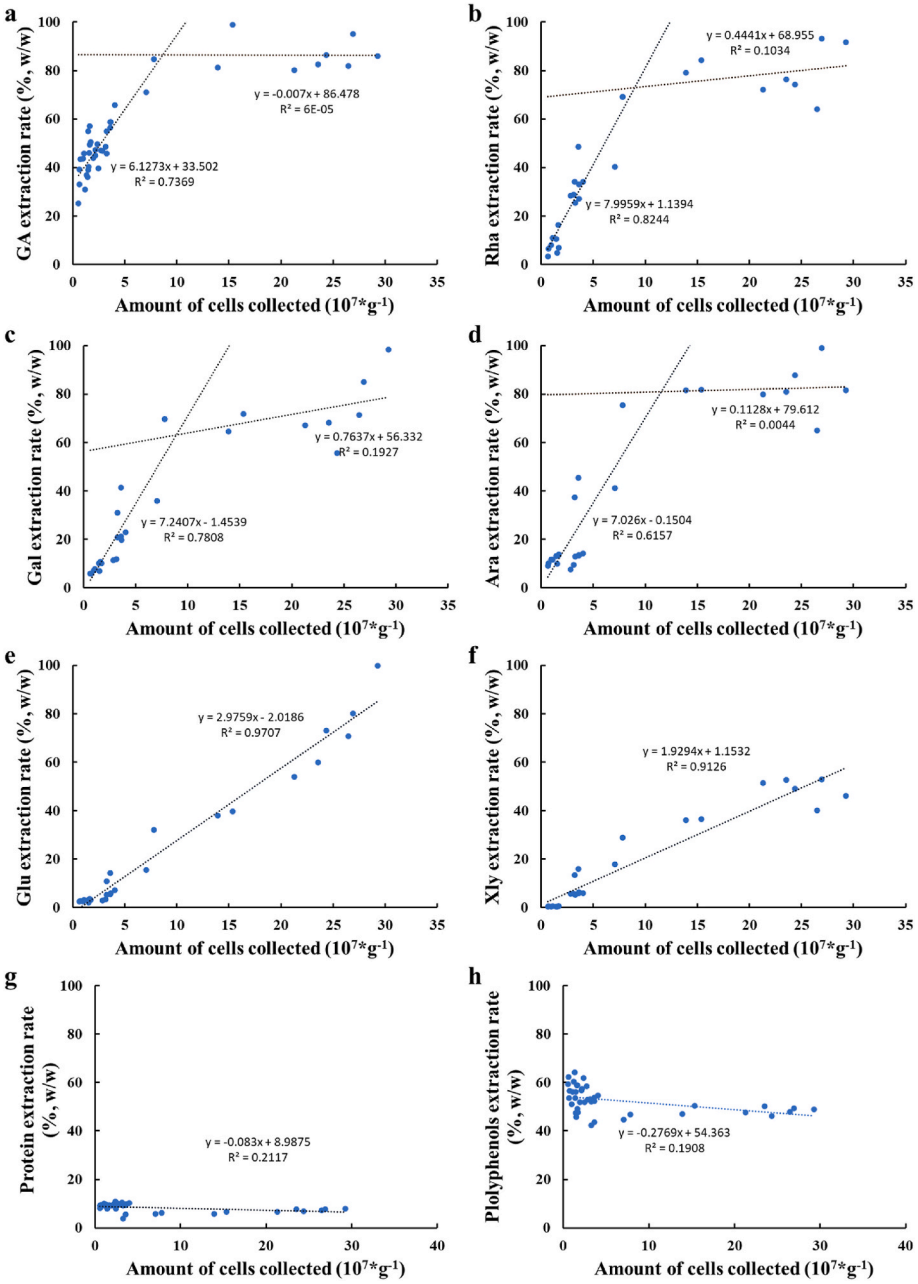

Fig. 5. Correlation between the extraction rate of soluble components and the number of collected mesophyll cells. a: galacturonic acid, b: rhamnose, c: galactose, d: arabinose, e: glucose, f: xylose, g: protein, h: polyphenols.

(30%). The higher protein content in collected cells could be attributed to the separation of leaf tissue parts with low protein content, such as epidermis and veins, and the dissolution of non-protein substances. When Celluclast® 1.5 L was used to degrade the mesophyll cell wall components, such as (hemi-) cellulose (C. Zhang et al., 2017), the highest protein content presented in collected cells was 50%. Ammonium oxalate and EDTA treatments only achieved protein recovery rates of approximately 12 and 7%, respectively, which resulted from the low yield of mesophyll cell collection. However, with the use of Celluclast® 1.5 L, a high collection of GTR mesophyll cells was achieved, with a protein recovery of 88%. The high protein content of the collected mesophyll cells achieved proved that collecting mesophyll cells is a viable solution for enriching the protein in GTR. In addition, the collected mesophyll can be further processed to produce Pickering emulsion (Ren et al., 2019), artificial meat (Wild et al., 2014) offering high-quality leaf protein for the food industry. This study provides a simple and novel process that is easily upscaled, which can be applied to not only tea leaf but also other leaves.

SEM was used to examine the fine structure of the collected mesophyll cells, and the results are shown in Fig. 5b. The mesophyll cells collected through ammonium oxalate and EDTA treatment exhibited a film-like structure in their outer layer, which was not observed in the mesophyll cells collected via Celluclast® 1.5 L treatment. This observation further confirmed that the significant amount of dissolved polysaccharides originated from the lamella layer and the cell wall during the dispersion of mesophyll cells with the Celluclast® 1.5 L treatment.

4. Conclusions

In this study, Celluclast® 1.5 L treatment had a better performance of leaf tissue dispersion and higher protein recovery than EDTA and ammonium oxalate treatments. The maximum mesophyll cells obtained

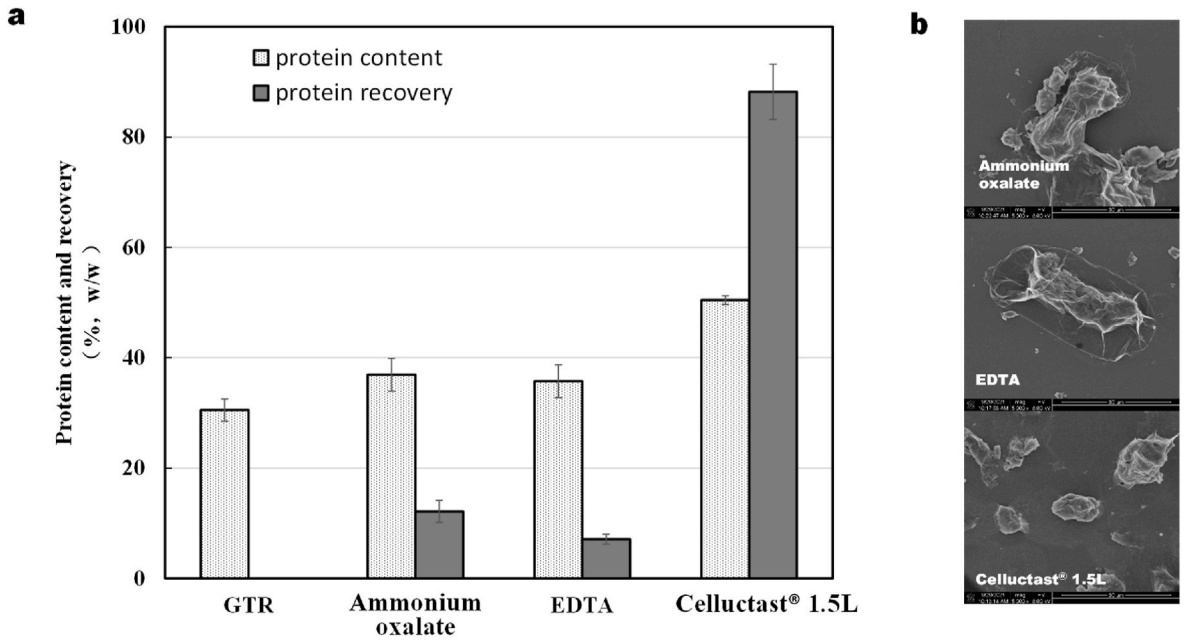

Fig. 6. Protein content in GTR and mesophyll cells collected using 0.1 M EDTA at 60 °C, 0.1 M ammonium oxalate at 60 °C, and 0.02 M acetic acid-sodium acetate buffer (pH 4.5) containing 1.2 U Celluclast® 1.5 L at 40 °C, with their corresponding protein recovery rates (a), and SEM images of collected mesophyll cells (b).

via Celluclast® 1.5 L treatment at 40 °C for 15 h was about 2.7×10^6 g^{-1} of GTR, which was about 7 times higher than that obtained through ammonium oxalate, and about 12 times higher than that obtained via EDTA. Celluclast® 1.5 L treatment enriched protein content 1.65 times in the collected mesophyll cells to 50% with a protein recovery of 88%. Our findings show that the degradation of cellulose and hemicellulose is the key to the dispersion of mesophyll, and the degradation of the primary cell wall can enhance the enrichment effect of the GTR protein.

CRediT authorship contribution statement

Chen Zhang: Conceptualization, Writing – original draft, Resources, Supervision. **Ziyang He:** Validation, Formal analysis, Writing – review & editing. **Ankun Wang:** Writing – review & editing. **Feipeng Zhang:** Methodology, Formal analysis, Investigation, Writing – original draft.

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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Data availability

Data will be made available on request.

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