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Improving yield and composition of protein concentrates from green tea residue in an agri-food supply chain: Effect of pre-treatment

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ABSTRACT

Rather than improving crop-production yield, developing biorefinery technology for unused biomass from the agri-food supply chain may be the crucial factor to reach sustainable global food security. A successful example of food-driven biorefinery is the extraction of protein from green tea residues, however, alkali usage is high and the resulting low protein quality limits its application. The research objective was to investigate the influence of pre-treatments with ethanol, Viscozyme® L and/or H_2O_2 on the subsequent alkaline protein extraction, and on their possible products for food applications. Polyphenols and/or pigments can be obtained by ethanol pre-treatment. Galacturonic acid and glucose can be obtained using Viscozyme® L. Pre-treatments using ethanol or Viscozyme® L individually reduced alkali consumption by 25% and improved protein extraction yield and purity. Their combination has the best effect. Additionally, pre-treatment using 50% ethanol reduced browning by 59% while pre-treatment using Viscozyme® L increased contents of arginine, threonine, and serine in the final alkaline protein extract. H_2O_2 pre-treatment had a negative effect on the alkaline protein extraction. These pre-treatments and protein extraction can be added to the existing process.

1. Introduction

"Twice the food production at half the ecological footprint" is the EU goal for 2050 that combines the ambition for less human impact on the environment with the rapidly growing world population and living standards (Alexandratos and Bruinsma, 2012; Hontelez, 2010). Rather than improving crop-production yield, developing biorefinery technology for unused biomass from the agri-food supply chain may be the crucial factor to reach sustainable global food security (Ranganathan, 2013). Residues from food industry have very specific advantages that make them especially suited as cheap and easy starting materials for biorefinery. First, they are already collected and therefore costs on transportation are limited. Secondly, they have often been pre-treated in the factory. This can either be an advantage or a disadvantage as pre-treatment may make components more accessible, but may also cause degradation of valuable components. The last important point is that food driven biorefinery can add a new outlet within the context of an existing process and product outlet, which is already cost competitive. This is in contrast development of entirely new biorefinery concepts, where new technologies are combined with multiple new outputs. A successful example of food-driven biorefinery is the extraction of

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protein from green tea residues (GTRs). These residues are left after tea lemonade production, which are and are currently at best used for energy through burning. A cost efficient method using alkali for protein extraction was previously developed (Zhang et al., 2014a).

However, the overuse of chemicals leading to generation of large amounts of salts, and the subsequent low protein quality in terms of protein content, taste, digestibility, and nutritional value, limits its applicability (Zhang et al., 2014a). During extraction, alkali first reacts with buffering components without changing the pH, before it can be used to increase pH needed for protein extraction *n* (Zhang et al., 2014a, 2015). The required alkali amount may be reduced when these buffering components are removed prior to protein extraction. Quality decrease of leaf protein in alkaline extracts may be due to the reaction of pigments, polyphenols, carbohydrates, or lignin with protein, which generates bitterness and decreases protein digestibility (de Toledo et al., 2013; Felicetti and Schrader, 2009; Rubanza et al., 2005; van Soest and Mason, 1991). These reactions can be quantified by the degree of browning, as the reaction products result in a brown colour of protein products under alkaline conditions (Felicetti and Schrader, 2009; Ozdal et al., 2013; van Soest and Mason, 1991). Browning can be accelerated when high temperature or pH are applied (Carvalheiro et al., 2008). Removal or prevention of colouring components before alkaline protein extraction may improve protein quality.

Many chemicals and/or enzymes can be used as pre-treatments to remove pigments, polyphenols, carbohydrates, and lignins from leafy biomass. Solvents (ethanol, acetone, hexane) are used for isolating pigments and polyphenols (Turkmen et al., 2006). Cell wall degrading enzymes, such as Viscozyme® L, pectinase, and Celluclast® are used to enhance protein extraction by degrading cell wall polysaccharides, such as pectin, and (hemi-) cellulose (Bals et al., 2007; Jodayree et al., 2012; Rosset et al., 2014). H_2O_2, which breaks down lignin, is also used to increase the dry matter digestibility of biomass used as animal feed (Chaudhry, 1998; Mishra et al., 2000). All these pre-treatments can be carried out at mild conditions ($T < 60\,^{\circ}C$), preserving the integrity of other side products such as pigments, polyphenols, and carbohydrates.

Pre-treatments using ethanol, Viscozyme® L, and/or H_2O_2 may fit in an integrated bio-refinery concept for leafy material (Zhang et al., 2015), but three issues need to be addressed to get from a concept to a process design. First, efficiencies of the pre-treatments on isolating pigment, polyphenol, carbohydrate, and/or lignin from biomass. Second, the influence of these pre-treatments on the efficiency of a subsequent alkaline protein extraction in terms of alkali consumption, protein yield, and protein quality (including appearance, nutrient digestibility, and nutritive value). Third, the assessment of these processes for the integration with the existing process of tea lemonade production. To address these issues, we tested ethanol, Viscozyme® L, and/or H_2O_2 as pre-treatments for alkaline protein extraction of GTR. The yield of chlorophyll, carotenoid, polyphenol, carbohydrate, as well as the protein content, were measured to analyse the extraction efficiencies of these pre-treatments and their possible products. Alkali consumption, protein yield, protein purity, colour formation, and dry matter digestibility for animal feed (Jayasuriya et al., 1978; Wu et al., 2014) were measured as efficiency indicators of these pre-treatments on alkaline protein extraction and as a measure of protein quality. Based on the results, the possibility of the integrated process for the biorefinery of GTR in tea lemonade factories is discussed.

2. Materials and methods

2.1. Materials

Green tea residue (GTR) is a gift from Damin Foodstuff (Zhangzhou) Co., Ltd., Fujian Province, China. This residue from tea lemonade production was collected from Camellia sinensis trees in Zhejiang province, China in 2014, and was sun-dried after soaking the green tea leaves in water at 85 °C for 45 min.

Viscozyme® L (Multi-enzyme complex containing a wide range of carbohydrases, including arabanase, cellulase, β-glucanase, hemicellulase, and xylanase) was purchased from Sigma–Aldrich, St. Louis, MO, USA If not stated otherwise, other chemicals used for analysis were purchased from Sigma–Aldrich, USA and of analytic grade.

2.2. Pre-treatments and protein extraction

2.2.1. Ethanol pre-treatment

To optimise ethanol extraction efficiency, GTR (200 mg) was first soaked in 4 mL 0–100% (v/v) ethanol, and incubated in a Thermomixer (VWR International B.V., USA) at 60 °C and 1000 rpm for 2 h. In subsequent experiments, GTR (200 mg) was soaked in 4 mL 50% ethanol or absolute ethanol in 10 mL tubes, and then incubated in a Thermomixer (60 °C, 1000 rpm) for 1, 5, 15, 30, 60, or 90 min. After centrifugation (7,000 g, 20 °C, 10 min), liquid extracts were collected and stored at 4 °C until further analysis.

2.2.2. Viscozyme® L pre-treatment

GTR (200 mg) was suspended in 4 mL 0.02 M sodium acetate–acetic acid buffer at pH 4.5, and then mixed with Viscozyme® L of 0, 12, 30, 60, or 120 FBGU (Fungal betaglucanase units)/g GTR activity, and supplemented to 4.2 mL with demineralised water. After incubation in a Thermomixer (30 °C, 1000 rpm) for 20 h, 4 mL demineralised water or ethanol was added to the mixtures, and then centrifuged (7000 × *g*, 20 °C, and 10 min). The liquid extracts were collected and stored at 4 °C until further analysis.

2.2.3. H_2O_2 pre-treatment

GTR (200 mg) was suspended in 4 mL H_2O_2 solution at a concentration of 0, 0.5, 1, or 2% (v/v). After incubation in a Thermomixer (60 °C, 1000 rpm) for 2 h, 4 mL demineralised water or ethanol was added to the mixtures, and then centrifuged (7000 × *g*, 20 °C, and 10 min). The liquid extracts were collected and freeze-dried for the removal of excess H_2O_2. Dried samples were store at room temperature until further analysis.

2.2.4. Alkaline protein extraction

The pellets obtained after pre-treatment were washed with 4 mL fresh solution, corresponding to the extraction liquid, and used for a subsequent alkaline protein extraction. The alkaline protein extraction protocol was based on our previous research (Zhang et al., 2014a). Pipetting 7 mL 0.1 M NaOH to the pre-treated GTR, the mixture was homogenised and incubated in a Thermomixer (95 °C, 1000 rpm) for 2 h. After centrifugation (7000 × *g*, 20 °C, 10 min), protein extracts were collected and stored at 4 °C until further analysis.