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Critical parameters in cost-effective alkaline extraction for high protein yield from leaves

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ABSTRACT

Leaves are potential resources for feed or food, but their applications are limited due to a high proportion of insoluble protein and inefficient processing. To overcome these problems, parameters of alkaline extraction were evaluated using green tea residue (GTR). Protein extraction could be maximized to 95% of total protein, and, after precipitation by pH adjustment to 3.5, 85% of extracted protein was recovered with a purity of 52%. Temperature, NaOH amount, and extraction time are the protein yield determining parameters, while pH and volume of extraction liquid are critical parameters for production cost. The cost of energy and chemicals for producing 1 t GTR proteins is minimized to 102€, and its nutritional value is comparable to soybean protein. Furthermore, this technology was successfully applied to other sources of biomass and has potential to be used as a part of an integrated bio-refinery process.

1. Introduction

Leaf protein has been regarded as an additional protein source since 1960s [1–3]. These proteins can be used in food [3,4], animal feed [5,6], or when hydrolyzed to amino acids for other applications, such as bulk chemicals [7]. Tea leaf residue is one example of a potential new protein source. As a major agro product in China, 1.6 Million tonnes (dry weight) of tea leaf products were produced in 2011 [8]. Tea producers estimate that around one fifth (dry weight) of the tea residues are produced centrally and can be collected from instant tea factories [9].Tea residues, which are the waste of tea leaves after hot water extraction, contain 20–30% protein [10,11]. There are at least seven different types of protein, including Rubisco and glycoproteins [11–13]. The superior quality of tea leaf protein in terms of amino acid profile compared to soy bean meal has been documented [11].

However, although huge economic potential lies in leaf protein, its applications are severely impeded by low cost-efficient production. Protein extraction yield is relatively low that varies from 15% to 60% of total protein, depending on species and processing methods [6,14–16]. Furthermore, protein production yields are reduced during recovery, particularly for those processes, such as alkaline extraction, that generate protein hydrolysates [15,17]. Conventional alkaline extraction, has already been studied decades ago, but no significant improvement was made in leaf protein extraction. Despite the lower cost, alkaline extraction has the lowest profit among all extraction techniques for leaves primarily

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due to low protein yield [17]. If protein yield can be increased without increasing cost for extraction, the economic value of leaf protein can be exploited.

The low productivity of alkaline extraction may result from overlooking two points. Firstly, applying high temperature in alkaline extraction has shown to increase protein yield in some cases [18], which is conflicting with the general knowledge that heating results in protein precipitation. Secondly, the influence of solution to raw material ratio (v/w) and alkaline concentration (pH) on protein yield were always studied independently [11,19,20], but the influence of alkali amount, which is determined by both v/w and alkaline concentration, was never considered.

In this study, the possibility to increase protein yield at elevated temperature was investigated, followed by an evaluation of the influence of v/w and alkali amount on protein extraction yield. The parameters that involves in alkaline extraction were grouped to protein yield related and cost related, and its economic value was estimated. In addition, the general applicability of new parameter setup was tested by using other materials, like oolong tea residue, grass, and barley straw.

2. Materials &Methods

2.1. Materials

Green tea residue (GTR) is our main material, which is a gift from Damin Company, Fujian Province, China. This residue from tea lemonade production was collected from *Camellia sinensis* trees in Zhejiang province, and it was sun-dried after soaking green tea leaves in water at 85 °C for 45 min. The dried residue was then ground into powder. Its protein content is 26.5%, which was determined by the method of Kjeldahl [21].

Oolong tea residue (leaves collected from the *C. sinensis* trees in Fujian province, processed by Damin company, Fujian, China), and Barley straw (*Hordeum vulgare* L., from Cargill B.V., the Netherlands) were sundried, collected, and stored at room temperature for further use. Grass (Poa pratensis, from Wageningen, The Netherlands) was freshly harvested and used immediately.

NaOH, HCl, and other chemicals for analysis were of analytical grade, purchased from Sigma, the USA.

2.2. Protein extraction

Protein extraction was performed by soaking 0.5 g GTR in alkaline solution. NaOH concentration (0–0.1 M), temperature (25–95 °C), extraction time (5 min–24 h), and v/w (8–60 ml g^{-1}), were varied. After subsequent centrifugation, which was always performed at 15,000 g for 10 min (Sorvall centrifuge, Thermo Fisher Scientific, the USA), supernatants were collected and stored at −20 °C until further analysis.

In two-step protein extractions, 0.5 g GTR was first extracted with 0.1 M NaOH at v/w of 40 ml g^{-1} and 40 °C for 4 h. After centrifugation, the supernatant was obtained and stored at −20 °C until further analysis while the precipitate was then soaked in 0.1 M NaOH at v/w of 40 ml g^{-1} and 95 °C for 2 h. The supernatant from the second extraction and final cake were separated by centrifugation and stored at −20 °C until further analysis.

All the experiments were performed in triplicate, and the errors were calculated using standard deviation.

2.3. Protein precipitation

Protein supernatants obtained from two experiments: 1) 0.1 M NaOH at v/w of 40 ml g^{-1} and 40 °C for 4 h, and 2) 0.1 M NaOH at v/w of 40 ml g^{-1} and 95 °C for 4 h, were concentrated to 5 g protein/L by a rotary evaporator (IKA, Labortechnick, Germany). The pH of each sample (10 ml) was adjusted to pH 3–5, by the addition of 1 M HCl, and left still at 4 °C for 24 h. Protein precipitates were collected by centrifugation and stored at −20 °C until further analysis.

All the experiments were performed in triplicate, and the errors were calculated using standard deviation.

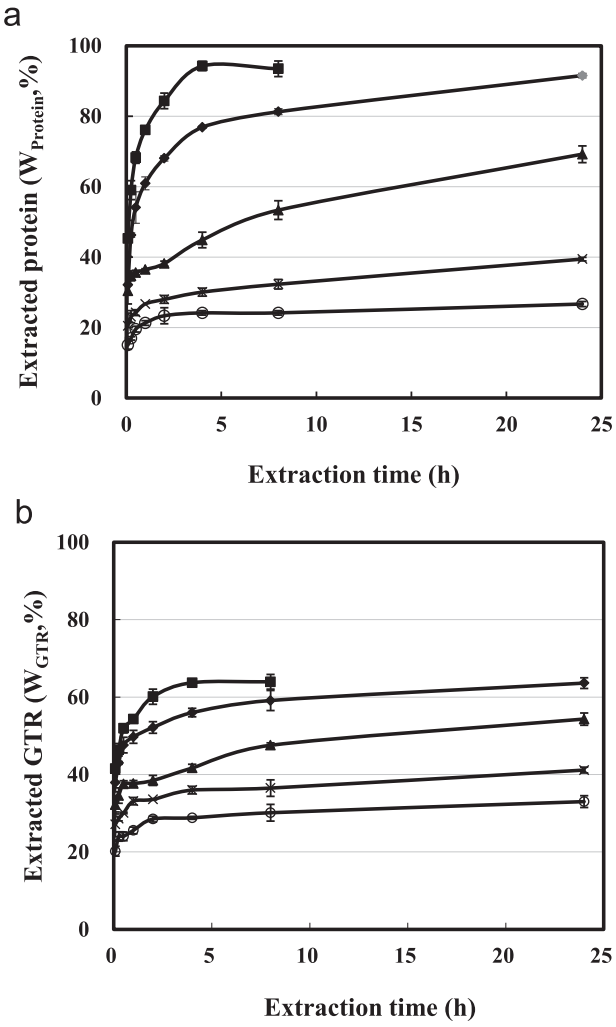

Fig. 1 – a) Protein extraction yield (WProtein,%) with 0.1 M NaOH and v/w of 40 ml g−1 at different temperatures: ■ 95 °C; ◆ 80 °C; ▲ 60 °C; × 40 °C; ○ 25 °C. b) Total mass extracted from GTR (W_{GTR}, %) with 0.1 M NaOH and v/w of 40 ml g^{-1} at different temperatures: ■ 95 °C; ◆ 80 °C; ▲ 60 °C; × 40 °C; ○ 25 °C.