〈Brief Note〉

Development of experimental teaching material for high school students: analysis of the protein content in Japanese green tea, black tea, and toasted tea

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Summary We have developed a method for the simple and rapid (within 60 min) quantitative analysis of protein concentration in different types of tea leaves such as green tea, black tea, and toasted tea. The optimized protocol follows three steps. First, 2 g of tea leaves are incubated at 90°C for 5 min in 100 mL water. Second, the supernatant obtained from the first step is filtered through a cartridge filter (0.45 μm). Third, the protein concentration in the filtered sample is measured using Bradford assay. Several experimental teaching protocols exist for high school students; however, they do not provide enough content. Therefore, the method was developed as an experimental teaching material for high school students towards preparing for higher pharmaceutical school. The protein concentration of green tea, black tea, and toasted tea was found to be 0.305 ± 0.023, 0.544 ± 0.030, and 0.264 ± 0.026 mg/mL, respectively (Mean ± SD).

Key words: Tea leaves, Teaching material, Protein concentration

1. Introduction

Tea is one of the most popular beverages consumed worldwide. There are many different ways to categorize teas, such as green, toasted, or black teas. One of the ways is based on the levels of fermentation. Green and toasted teas are unfermented, whereas black tea is fully fermented. Japanese green tea such as gyokuro, sencha, and bancha are prepared by a steaming process whereas the toasted teas are prepared by a strong roasting process. Numerous studies have been published on tea and its health benefits on the lipid profile[1], blood

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glucose levels[2], cancer[3,4], hypertension[5,6], and weight reduction[7,8]. Green tea extracts specifically mediate Angiotensin II-induced hypertrophy through the inhibition of reactive oxygen species production by blunting a feed-forward mechanism involving the Src/EGFR/Akt signaling pathway and NAD(P)H activity[9]. The protein present in green tea also inhibits the activity of angiotensin-converting enzyme. The understanding of protein assay is important as first step for high school students in their gradual study of the functions of proteins in tea. In this study, the Bio-Rad Protein Assay, based on the method of Bradford[10] was used. This assay uses the colorimetric changes of a dye upon protein-binding. The absorbance maximum for an acidic solution of Coomassie brilliant blue G-250 dye shifts from 465 nm to 595 nm when bound to protein. The Coomassie blue dye primarily binds to basic and aromatic amino acid residues, especially arginine.

We have developed many methods as experimental teaching materials for high school students[11-15]. In this study, we have developed a method for measuring the protein concentration in different teas (green tea, black tea, and toasted tea). This method is simple (three steps), rapid (within 60 min), and accurate with calibration curve.

2. Materials and Methods

The samples of Japanese green tea (Hagiri Co., Ltd, Japan), black tea (Earl gray tea; Janet Paris Co. Ltd., France), and toasted tea (Nishikawaen Co., Ltd, Japan) were used in this study.

Bovine serum albumin was used as the protein standard. The effect of varying the temperature and incubation time on the concentration of protein extracted from the tea leaves was analyzed. The extracted samples were filtered through a cartridge filter (Millex LH 0.45 μm, Millipore, Tokyo, Japan). The protein content in these supernatants was measured by Bradford method using the Bio-Rad protein assay kit I (Bio-Rad, Herculten, CA, USA).

3. Results and Discussion

The protein concentration in the solutions prepared from 2 g tea leaves, incubated in 100 mL water for 5 min at 40°C, 60°C, 80°C, and 90°C, was 0.191 ± 0.023, 0.264 ± 0.022, 0.299 ± 0.073, and 0.387 ± 0.003 mg/mL, respectively (Mean ± SD, Fig. 1). Keeping the solution at a constant temperature higher than 90°C was difficult and was adjudged

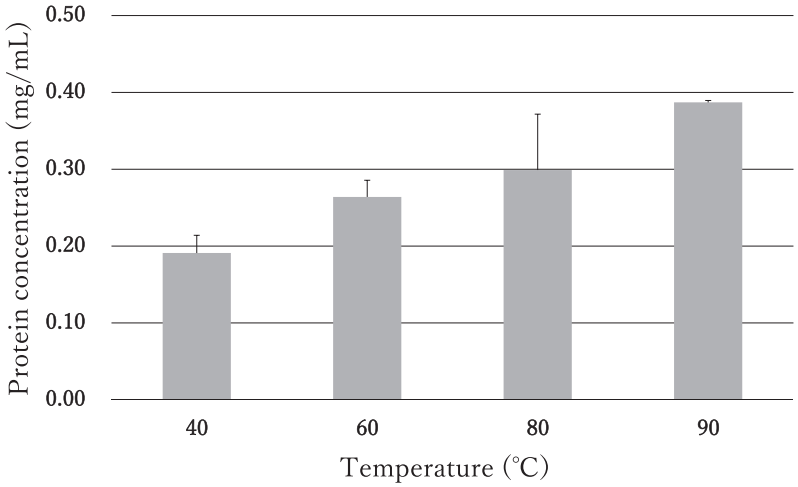

Fig. 1 The effect of increasing water temperature on protein concentration. Green tea leaves were incubated for 5 min in water of varying temperature ranging from 40°C to 90°C prior to protein extraction and determination of concentration using the Bradford assay. The data are presented as mean ± SD of three infusions per sample.

to be unsafe for high school students. The best temperature condition for extracting protein in teas was 90°C. At this temperature, the protein concentration in the solutions prepared from 2 g tea leaves incubated in 100 mL water for 2, 5, 10, 20, 30, and 40 min was measured (Fig. 2) and determined to be 0.233 ± 0.041, 0.305 ± 0.042, 0.336 ± 0.069, 0.331 ± 0.026, 0.359 ± 0.038, and 0.343 ± 0.018 mg/mL, respectively (Mean ± SD). There were no changes in protein concentration at treatment times longer than 5 min. However, at 5 min, the protein concentration was 1.3 times higher than that at 2 min. The best treatment condition, therefore, was found to be 5 min incubation at 90°C. Next, the effect of processing tea leaves by grinding in a mill-mixer on protein concentration was evaluated. Both types of green tea leaves were processed using a mill-mixer (Iwatani Co., Ltd, Japan) for 1.5 min and the protein concentration relative to that of unprocessed tea leaves was measured (Fig. 3). The protein

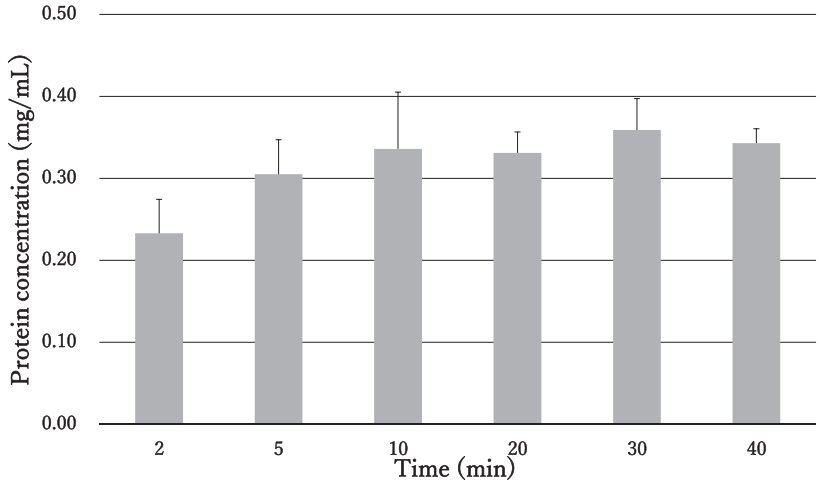

Fig. 2 The effect of increase increasing incubation time in water at 90°C on protein concentration. Green tea leaves were incubated in water at 90°C for 2, 5, 10, 20, 30 and 40 min prior to extraction and protein concentration analysis using the Bradford assay. The data are presented as mean ± SD of three infusions per sample.

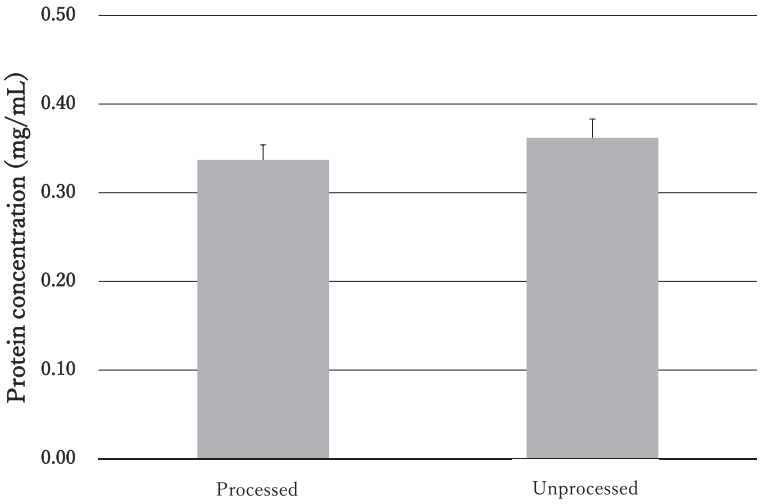

Fig. 3 The effect of processing tea leaves in a mill-mixer. Green tea leaves were incubated in water at 90°C for 5 min with or without 1.5 min processing in a mill-mixer prior to extraction and protein concentration analysis using the Bradford assay. The data are presented as mean ± SD of three infusions per sample.

concentration in the solution of tea leaves processed with the mill-mixer was 0.337 ± 0.017 mg/mL, as compared to 0.362 ± 0.021 mg/mL for the unprocessed sample (Mean ± SD). These data showed no significant differences between the two methods. Therefore, the simpler and easier alternative of using unprocessed tea leaves was preferred to using tea leaves processed in a mill-mixer. In summary, the best conditions for extracting protein from leaves of green tea were found to be incubating unprocessed leaves (2 g) in 100 mL water at 90°C for 5 min. This procedure is simple, easy, and takes short time to prepare (<10 min). This method was then used to compare the protein concentration of green tea, black tea, and toasted tea, which was determined to be 0.305 ± 0.023, 0.544 ± 0.030, and 0.264 ± 0.026 mg/mL, respectively (Mean ± SD; Fig. 4). It has been previously reported that the amount of protein in green tea leaves is 25-30% of dry mass[16] using the Kjeldahl method[17]. This ratio is the highest in the tea leaves. In this study, the amount of protein in 2 g tea leaves extracted in 100 mL water was determined using the Bradford assay. There was, therefore, a clear difference in protein concentration between the Kjeldahl method and our method possibly due to differences in the efficiency of protein extraction from the tea leaves to solution. The functions of several proteins present in the tea leaves may be elucidated in future. In this study, the protein concentration of black tea was determined to be the highest, while that of the toasted tea was found to be the lowest of three types of tea leaves analyzed. The fermentation process used to produce black tea may be responsible for the increase in protein content in this tea, but toasting process used in making toasted tea may decrease the protein content. Further studies in this regard are in progress.

In conclusion, we have developed experimental teaching materials for high school students. This method is easy, simple, and takes short time to perform (<60 min). This method can be applied for effective teaching in high school lessons because the protein assay is included in the model core curriculum for pharmacy education[18] in chapters C2(1): fundamentals of analytical methodology, C2(3): qualitative and quantitative analyses of chemical substances, C2(4): instrumental analysis, C6(2): fundamentals of biomolecules, and C6(3): proteins responsible for biological functions. The model core curriculum for pharmacy education plays a major role in ensuring that pools of pharmacists and pharmaceutical researchers are available by improving the quality of pharmacy education offered and meeting social responsibilities to uphold the highest standards for their qualification. This developed method is an important analytical technique for the

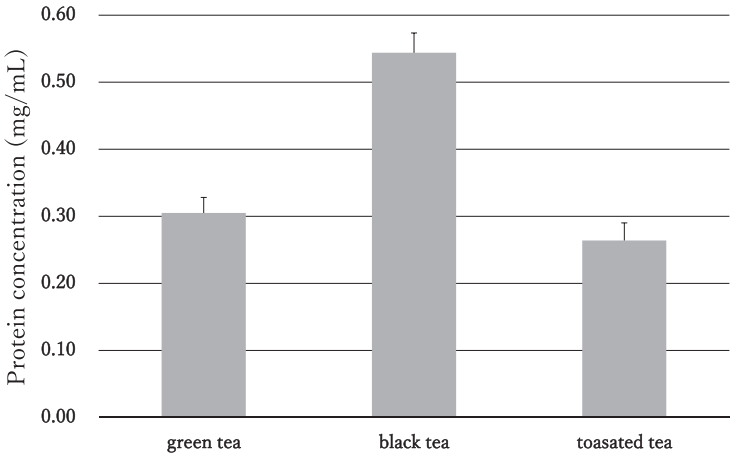

Fig. 4 Protein amount in water extracts of three kinds of teas: green tea (sencha), toasted tea, and black tea. Unprocessed tea leaves were incubated for 5 min in 90°C water prior to extraction and protein concentration analysis using the Bradford assay. The data are presented as mean ± SD of three infusions per sample.

pharmacists because pharmacists need to correctly understand case report forms containing blood and urine data. The principle of this study relates to the chapters, *Chemical Equilibria and Naturally Occurring Polymers*, in chemistry and the chapter, *Cell and Molecular*, in biology for high school.

Conflicts of interest

All authors have no conflicts of interest.

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References

1. Erba D, Riso P, Bordoni A, Foti P, Biagi PL, and Testolin G: Effectiveness of moderate green tea consumption on antioxidative status and plasma lipid profile in humans. J Nutr Biochem, 16: 144-149, 2005.
2. Maruyama K, Iso H, Sasaki S, and Fukino Y: The association between concentrations of green tea and blood glucose levels. J Clin Biochem Nutr, 44: 41, 2009.
3. Mu LN, Lu QY, Yu SZ, et al: Green tea drinking and multigenetic index on the risk of stomach cancer in a Chinese population. Int J Cancer, 116: 972-983, 2005.
4. Sun CL, Yuan J-M, Koh WP, and Mimi CY: Green tea, black tea and breast cancer risk: a meta-analysis of epidemiological studies. Carcinogenesis, 27: 1310-1315, 2006.
5. Antonello M, Montemurro D, Bolognesi M, et al: Prevention of hypertension, cardiovascular damage and endothelial dysfunction with green tea extracts. Am J Hypertens, 20: 1321-1328, 2007.
6. Nagao T, Hase T, and Tokimitsu I: A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. Obesity, 15: 1473-1483, 2007.
7. Auvichayapat P, Prapochanung M, Tunkamnerdthai O, et al. Effectiveness of green tea on weight reduction in obese Thais: a randomized, controlled trial. Physiol Behav, 93: 486-491, 2008.
8. Moher D, Liberati A, Tetzlaff J, and Altman DG: Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med, 151: 264-269, 2009.
9. Pappareallа I, Ceolotto G, Montemurro D, et al: Green tea attenuates angiotensin II-induced cardiac hypertrophy in rats by modulating reactive oxygen species production and the Src/epidermal growth factor receptor/Akt signaling pathway. J Nutr, 138: 1596-1601, 2008.
10. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 72: 248-54. 1976.
11. Okamoto M and Nishiguchi Y: Rapid measurement of caffeine in green tea with a UV/vis spectrometer. Med Biol, 155: 955-958, 2011.
12. Nishiguchi Y, Kitamura K, Goromaru-Shinkai M, Nakazawa K, Narusuye K, Ito N, and Nagahama T: Improvement of cell fusion from two types of protoplast, the leaf from petunia (*Petunia hybrid*) and petal from surfinia (*Petunia hybrid*) as teaching material in science education. Med Biol, 157: 22-26, 2013.
13. Goromaru-shinkai M, Nakazawa K, Shimizu M, Kuroda J, Nishiguchi Y, Nakahama T, and Akimoto Y: A simple experimental teaching material for radiation with educative effect. Toho Liberal Arts Review, 47: 1-9, 2015.
14. Nishiguchi Y, Goromaru-shinkai M, Kuroda J, Kiuchi S, and Ihara H: Estimation of protein,total polyphenol, chlorogenic acid, caffeine, and caffeic acid contents in Indonesian palmcivet coffee (*Kopi Luwak*). Int J Anal Bio-Sci, 5: 53-56, 2017.
15. Goroumaru-Shinkai M, Kuroda J, Nakazawa K, Narusuye K, Nishiguchi Y, and Ichiva H: Effect of cigarette extra against vitamin C contents of broccoli spraut. Int J Anal Bio-Sci, 7: 1-6, 2018.
16. Edited by Arai E, Arayama M, and Yanagisawa S: Visual wide, the table of food component, 234-335, Tokyo shoseki co., Ltd, Japan (2010).
17. Kjeldahl J: New method for the determination of nitrogen in organic substances. Zeitschrift Fur Analytische Chemie, 22: 366-383, 1883.
18. Ozawa K, Ichikawa A, Furusawa Y, et al.: Model core curriculum for pharmacy education-2015 version-, 24: 14-15, 2018.