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VISUAL EXPERIMENT

A Bradford multiplexing method for protein estimation in fermented foods: Soy sauces

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ABSTRACT

Proteins for foods, in addition to providing nutrition, should also possess specific functional properties that facilitate processing and serve as the basis of product performance. Soy protein is a major component of the diet of food and is increasingly important in the human diet. Hence, here in the present article, we are focusing a rapid and easy method for quantitative determination of total protein content with multiplex samples in any food products such as soy sauce or other traditional fermented foods. We described a bioassay procedure (Bradford method) for the evaluation of total protein content in foods. This method involves measurement of the protein efficiency ratio under standardized conditions. The experiment will provide researchers a scientific way to determine pretentious quality of variety of foods and/or health supplements.

INTRODUCTION

Soybean products are considered healthy foods, and especially fermented soybean products are important traditional foods in Asian countries such as Korea, China, and Japan. Dietary intake of fermented soy products is linked to reduced risk of chronic diseases such as cancer and cardiovascular diseases (Shon et al., 2007). The main source of diet is protein content from plants and animals. As a traditional seasoning, soy sauce is an important dietary component in Asian countries due to its intense savory taste and characteristic profile having caramel, roasted, and malted aroma (Feng et al., 2013).

Due to the critical role of soy sauce in diet and health, measuring the protein content of fermented food products is gaining more importance than ever. Food production companies need to monitor protein content at multiple points along the food process chain. There are several analytical methods available for the quantitative determination of protein content such as titration-based methods, chromatographic methods and spectrophotometric methods. Among them, the Bradford protein assay is one of the unique and simple methods commonly used to determine the total protein concentration of variety of samples. The Bradford reagent can be used to determine the concentration of proteins in solution. The procedure is based on the formation of a complex between the dye, Brilliant blue G, and proteins in solution. The protein-dye complex causes a shift in the absorption maximum of the dye from 465 to 595 nm. The amount of absorption is proportional to the protein present. The protein concentration of a test sample is determined by comparison to that of a series of protein standards known to reproducibly exhibit a linear absorbance profile in this assay. Although different protein standards can be used, we have chosen the most widely used protein, bovine serum albumin (BSA) as a standard drug in this study.

In the present visual experimental research, we step-by-step demonstrated how to quantify total protein content in a fermented product soy sauce with rapid and multiplexing way using Bradford assay which can be further used to determine protein content in any kinds of traditional and commercial fermented foods being used as nutritional health supplements.

MATERIALS AND EQIPMENTS

Disposables

Micropipettor tips

Eppendorf tubes (1.5 mL)

Syringe filters (0.45 µm)

96-well microtiter plate

Falcon tubes (15 mL)

Eppendorf tubes

Major reagents

Bovine serum albumin (BSA) (Sigma-Aldrich)

Bradford reagent (Sigma-Aldrich)

Distilled water

Traditional soy sauce samples (to be tested for total protein determination)

Equipments

Spectrophotometer (Tecan)

Vortex

Multichannel pipette

Solutions

Standard bovine serum albumin (BSA) solution

Measure 60 mg of standard BSA and dissolve in 20 mL of distilled water to make a final concentration of 3 mg/mL

REAGENTS AND SOLUTIONS

Table I

Dilution of bovine serum albumin (BSA) solution

Final concentration of BSA (mg/mL)	BSA volume (µL)	Distilled water volume (µL)
0.0	0.0	1,000
0.3	100	900
0.6	200	800
0.9	300	700
1.2	400	600
1.5	500	500
1.8	600	400
2.1	700	300
2.4	800	200
2.7	900	100
3.0	1,000	0

VIDEO CLIPS

Requirement and sample preparation method	3 min 26 sec
Assay procedure	6 min 4 sec
Measurement of absorbance using an ELISA microtiter plate reader	5 min 1 sec

METHOD

Preparations

1. Prepare a 96 well microtiter plate with proper marking on wells for accurate sample inoculation
2. Prepare a range of 10 concentrations of standard BSA ranged from 0.3-3.0 mg/mL using appropriately labeled Eppendorf tubes
3. Prepare soy sauce samples to be tested after filtering via syringe filters (0.45 µm)

[We can use any kind of food sample replacing soy sauce]

[If sample if solid, then we need to do extraction or grinding with appropriate solvent followed by filtration]

Assay procedure

1. Add 5 µL of standard solution and sample in a 96-well microtiter plate (in triplicates)
2. Add 250 µL of Bradford reagent in each well by using multichannel pipette
3. Mix the solution properly using up and down pipetting
4. Incubate the 96-well microtiter plate at room temperature in dark condition for 5 minutes
5. Measure the absorbance at 595 nm.

[Always consider to maintain a dark condition while handling Bradford reagent because of its rapid oxidization property]

DISCUSSION

Soy sauce is a traditional fermented condiment commonly consumed in China, Korea and Japan which is gaining world-wide popularity nowadays due to its various nutritional properties (Lioe et al., 2010). Studies have shown that soy sauce has a promotive effect on human health, such as on iron absorption in human subjects (Baynes et al., 1990). Soy sauce is a mixture of protein hydrolysate and carbohydrate materials made by the action of enzymes and microorganisms. The product contains amino acids, peptides, reducing sugars and flavor compounds which are formed during the process and which impart tasty flavor with multiple therapeutic and pharmacological actions (Lioe et al., 2010). Fermentation of soy sauce uses enzyme to hydrolyze proteins and starch, that results in the formation of certain amount of substances such as amino acids, and sugars. Several researchers reported 16-18%of total protein in commercially available soy sauce. Here in our present study, traditional samples of soy sauces showed similar protein contents which ranged from 10-15%. Therefore, in the present research, we developed and demonstrated a new methodology for determining the total protein content in soy sauce samples because the developed procedure has advantage of added feature of multiplexing which will certainly help to process a large amount of sample at the same time thus can be time saving and economical for both industrial and scientific purposes. The proposed protocol can be applicable on variety of food and/or clinical samples.

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PRECAUTIONS

Bradford reagent should be handled in dark condition in order to avoid unwanted oxidation

Before measurement of 96-well plate for absorbance, bubbles from each well should be removed for consistent data