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Analytical Methods

A practical method for extending the biuret assay to protein determination of corn-based products

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ABSTRACT

A modified biuret method suitable for protein determination of corn-based products was developed by introducing a combination of an alkaline reagent with sodium dodecyl sulfate (reagent A) and heat treatments. The method was tested on seven corn-based samples. The results showed mostly good agreement ($P > 0.05$) as compared to the Kjeldahl values. The proposed method was found to enhance the accuracy of prediction on zein content using bovine serum albumin as standard. Reagent A and sample treatment were proved to effectively improve protein solubilization for the thermally-dried corn-based products, e.g. corn gluten meal. The absorbance was stable for at least 1-h. Moreover, the whole measurement of protein content only needs 15–20 min more than the traditional biuret assay, and can be performed in batches. The findings suggest that the proposed method could be a timesaving alternative for routine protein analyses in corn processing factories.

1. Introduction

Corn is one of the most important crop produced worldwide. According to the latest statistics, China's corn production hit a record 224.58 million tons, and nearly one-quarter is processed to produce various products, such as starch, sweeteners, organic acids, fuels and feeds (Wang, 2016). The feed products including corn gluten meal, corn germ meal, distiller's dried grains and solubles, accounting for about 25 percent of the corn products, are utilized as important supplemental protein sources in animal diets. Protein determination of intermediate and end products as required by quality control management is a laborious routine work. Kjeldahl method and Dumas method are the international standard methods for the quantitative determination of crude protein in feed and food products based on digestion-titration and combustion, respectively. However, conventional Kjeldahl digestion is time-consuming (at least 1–2 h), and the Dumas has a high initial cost and the small sample size makes it difficult to obtain a representative sample. Furthermore, another disadvantage for both methods is a suitable nitrogen conversion factor depending on the type of protein is required (Dakin & Dudley, 1914; Figenschou, Marais, & De Figueiredo, 2000; Finete, Gouvêa, Marques, & Netto, 2013).

The biuret method, as a classical colorimetric method, is rapid and simple to carry out, and does not depend on the amino acid composition of the protein with a fine sensitivity. However, the accuracy achieved is sometimes not satisfactory for the thermally-dried corn-based products. Because drying with superheated steam or hot air may cause strong protein hydrophobic interactions and aggregations in the overheated portion, resulting in significant decreases of protein solubility and extraction efficiency (Malumba, Vanderghem, Deroanne, & Béra, 2008). Sodium dodecyl sulfate (SDS) can disrupt the interactions, but because SDS associates with potassium to cause a precipitation, it is not compatible with the traditional biuret reagent (Moore, DeVries, Lipp, Griffiths, & Abernethy, 2010; Watters, 1978). Therefore, sodium potassium tartrate in the biuret reagent could replace with sodium tartrate to improve the method, and extend the range of application (Watters, 1978). This modification was also adopted by the Markwell (a modified Lowry) and bicinchoninic acid (BCA) methods, which were based on biuret reaction but with higher sensitivity (Markwell, Haas, Tolbert, & Bieber, 1981; Walker, 2002). However, in our previous study, the low efficiency of protein extraction, and starch, fiber, oil and other nonprotein substances relating to a turbid assay solution were found to impede the application of the colorimetric methods to corn-based products. Furthermore, when compared with the biuret reaction, the Markwell and BCA reactions are dependent on the amino acid composition, and chromophores are unstable resulting in a large derivation in batch measurements. The biuret assay is thereby chosen and fur-

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ther developed in the present study to provide an alternative rapid protein determination method for corn or corn-based products.

2. Materials and methods

2.1. Materials

Seven samples of corn and corn coproduct were randomly collected from starch and ethanol plants. Thermally-dried corn gluten meal (CGlM), corn germ meal (CGeM) were obtained from COFCO Biochemical Industry Co., Ltd. Corn flour (CF) and distiller's dried grains and solubles (DDGS) were obtained from COFCO Bioenergy Co., Ltd. Cornflakes was obtained from C. Hahne Mühlenwerke GmbH & Co. KG. Food-grade powdery zein was obtained from Freeman Industries LLC. Bovine serum albumin (BSA) was obtained from Amresco (No. 0332). Other chemicals were of reagent grade obtained from Sinopharm Chemical Reagent Co., Ltd.

2.2. Preparation of reagents

A solution containing 2.0% SDS and 0.8% sodium hydroxide was prepared and named as sample treatment reagent (or reagent A). A modified biuret reagent containing 0.15% cupric sulfate pentahydrate, 0.49% sodium tartrate dihydrate and 0.75% sodium hydroxide was prepared according to Watters (1978) and named as Watters reagent (or reagent B) (Watters, 1978). The reagents can be stable for at least 3-month when stored in plastic bottles at room or refrigerator temperature.

2.3. Sample treatment

Samples (except for zein and CF) were ground with a pulverizer (Great Wall GW-400, Shanghai, China) at a speed of 28000 rpm for 0.5–1 min followed by passing through a 60-mesh (or smaller) sieve. Then, the sieved samples were dispersed in distilled water to obtain suspensions containing approximate 0.2–16 mg/mL protein. Before being subjected to protein assay, the particle size of sample granules in solution could be optionally further reduced by a homogenizer (IKA T25) at 6500 rpm for 1 min. Aliquots (1 mL) of the well-mixed suspensions were transferred into screw-top test tubes (with cap), then equal volumes of sample treatment reagent were added to reach about 0.1–8 mg/mL protein. The mixtures were heated in a boiling water bath for 10–15 min. During heating, vigorous vortexing was applied at 3 min intervals for 5–10 s. The heated mixtures with colloidal appearance (Fig. 1) were obtained and quickly cooled down to room temperature in water.

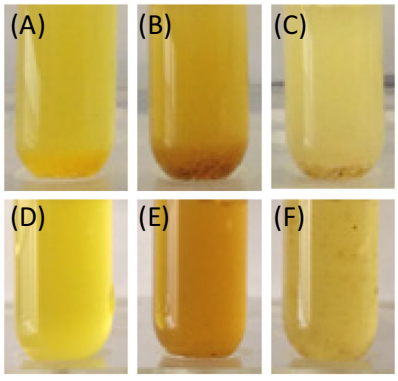

Fig. 1. Typical samples in reagent A before and after heat treatment. Samples of corn gluten meal (CGlM) (panel A and D), distiller's dried grain with solubles (DDGS) (panel B and E) and corn flour (CF) (panel C and F) were selected to present after mixed well with sample treatment reagent (A)–(C) and subsequentially heated in a boiling water bath for 10 min (D)–(F).

2.4. Protein assay

Aliquots (0.5 mL) of the cooled samples were mixed with 2.5 mL of Watters reagent. Because the use of detergent was reported to slow down the rate of color development (Watters, 1978), the incubation was carried out at 60 °C for 25 min. The colored samples were cooled to room temperature using a water or ice bath, and centrifuged at 1500*g* for 5 min to remove the insolubles. In the present method, when compared to Watters (1978), the concentration of SDS in the colored solution was reduced to 0.16% (about 6 mM) to give a better clarity, and corn proteins including zein were still soluble (Deo, Jockusch, Turro, & Somasundaran, 2003). The supernatants were filtered with 0.45 μm poly(ether sulfone) (PES) membrane, and the absorbances of filtrates were measured on an Unico Model 2800 UV/VIS spectrophotometer at 540 nm against a blank containing 0.5 ml of 1% SDS/0.4% sodium hydroxide and 2.5 ml of Watters reagent. In addition, because lipid could yield a cloudy reaction mixture, this substance was removed by addition of at least 0.6 ml petroleum ether and with thorough mixing before the centrifugation step, then the aqueous phase was subjected to filtration. To prepare standard curve, BSA, in amounts from 0.05 to 4 mg were dissolved in 0.5 ml of solution containing 1% SDS/0.4% NaOH, i.e. two-fold dilution of sample treatment reagent. Then the absorbance readings were obtained as described above. The protein concentration of each unknown sample was calculated by the standard curve with dilution factor.

When compared with the classical biuret assay, the proposed method introduced a preliminary protein extraction of the investigated materials with the Watters reagent-compatible solution (reagent A) and simultaneous heating treatment to effectively improve the solubility of proteins in the thermally processed corn products. Moreover, the incubation temperature during color development was increased to 60 °C. These modifications also brought about an enhanced accuracy of prediction on zein content using BSA as standard. The features were proved and elaborated below.

Kjeldahl nitrogen values were determined by the method of the AOAC. (2006). Protein contents were converted from the Kjeldahl nitrogen by the factor 6.24.

2.5. Evaluation of sample treatment in absorbance response

BSA (a standard protein) and zein (the major protein in corn) were individually dissolved in reagent A in the range of about 0.1–8 mg/mL with or without (as controls) heating, and the protein concentrations were determined by the proposed biuret method. In addition, the color stability of the proposed method was compared with that of bicinchoninic acid (BCA) method.

2.6. Evaluation of protein extraction

CGlM samples (with protein content of ~60%) were used as representative samples to investigate the effect of the proposed protocol on protein extraction. The samples were dispersed in distilled water at different protein concentrations of about 1 mg/mL, 4 mg/mL and 16 mg/mL. The following procedures were performed as described in Sections 2.3 and 2.4 until transparent filtrates were obtained, except that cupric sulfate pentahydrate was removed from Watters reagent. The protein concentrations were determined by the proposed method. Protein yields were calculated by dividing the biuret protein contents of filtrates by that of the solutions before centrifugation.