Misuse of the Kjeldahl Method for Estimating Protein Content in Plant Tissue

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Accurate plant protein analysis is difficult, primarily because of the small amounts of proteins present in the tissue and partly due to the interference of other complex plant constituents (Agustí and Beltrán, 1982; Khanizadeh et al., 1989, 1992a; Marks et al., 1985; Robinson, 1979). Many procedures have been used for protein determination in plant tissues. The most commonly used procedures for total protein estimation are: folin phenol (Lowry et al., 1951), biuret (Gornall and Bardawill, 1949), 280/260 nm ultraviolet (UV) absorption, improved 224–236 nm isoabsorbance (Groves et al., 1968), 280/205 nm absorption (Scopes, 1974), coomassie blue dye binding (Bradford, 1976), and classical Kjeldahl [Association of Official Analytical Chemists (AOAC), 1984; Morris, 1983]. However, some of these methods (e.g., folin phenol, Bradford and UV absorption, and biuret) cannot be used for plant tissue due to the presence of numerous interfering compounds, such as phenolics, tannins, and alkaloids (Benedict, 1987; Marks et al., 1985; Peterson, 1983; Robinson, 1979). Others, such as the spectrophotometric method of Warburg and Christian (1942) and Waddell and Hill (1956), cannot distinguish between protein and plant nucleic acid content (Groves et al., 1968). The biuret reaction is also subject to ammonia, tris, and glycerol interference (Bradford, 1976). Peterson (1983) examined various methods of protein determination based on their relative sensitivity for bovine serum albumin and a range of proteins. He concluded

that all spectrophotometric methods are similar in sensitivity, but more sensitive than biuret, 280-nm UV absorption, or turbidimetric methods. He also reported that procedures that are simple to perform usually give relative rather than absolute protein quantitations. In general, he suggested reliable estimates of the absolute amount of protein may be obtained by quantification of total amino acids. Agustí and Beltrán (1982) compared the three spectrophotometric methods of Lowry et al. (1951), Bradford (1976), and Flores (1978), and used Kjeldahl as a reference. They measured higher amounts of proteins by using the three spectrophotometric methods than by using the Kjeldahl procedure.

Of the above methods, Kjeldahl analysis and subsequent estimation of protein by multiplying total N by 6.25 is the most frequently used procedure, owing to its simplicity and low cost. Workers assume that all plant proteins have a mean N content of 16%. However, the Kjeldahl method does not distinguish between protein N, free amino acid N, or other nitrogenous compounds, such as inorganic N, alkaloids, cyanogenic glycosides, amides, ammonium salts, nitrogenous glucosides, porphyrins, fats, hormones, nucleic acids, etc. Therefore, the conversion factor of 6.25 is inexact and results in overestimation of total protein in most plant tissue (Khanizadeh et al., 1992b; Milton and Dintzis, 1981). We believe that a specific conversion factor should be calculated for each tissue to estimate total protein content accurately, given that total nonprotein N content varies from plant to plant, between different tissues on the same plant, and even in the same tissue at different stages of growth and development (Jones, 1931, 1991; Jones et al., 1991; Khanizadeh et al., 1989; Milton and Dintzis, 1981). While the factor of 6.25 is widely used for many plant tissues, specific factors for total protein determination have been developed for many crops. For example, 5.71 for wheat-flour (*Triticum aestivum* L.), 5.18 for almonds (*Prunus amygdalus* Batsch.), 5.46 for peanuts (*Arachis hypogaea* L.) and Brazil nuts (*Bertholletica excelsa* Humb. and Bonpl.), 5.3 for tree nuts and coconuts (*Cocos nucifera* L.), 4.4 for tropical plant samples, and 5.51 for apple (*Malus pumila* Borkh.) flower bud tissue, etc. (AOAC, 1980, 1984; Benedict, 1987; Heidelbaugh et al., 1975; Jones, 1931; Khanizadeh et al., 1992b; Milton and Dintzis,

1981). Jones (1931) proposed individual conversion factors for many plants and food products.

Several techniques have been proposed as alternatives to the Kjeldahl procedures: 1) Dumas combustion, which determines total N (Hansen, 1989); 2) near-infrared reflectance spectrometry, which gives results similar to the Kjeldahl procedure, since the method is based on the approximate relationship between N and protein content (Jones, 1991); and 3) amino acid analysis using the ninhydrin reaction in which the protein content is measured by total amino acid composition using ion exchange columns (Horstmann, 1979; Khanizadeh et al., 1992b). The estimation of total protein is based on the summation of amino acid N after breaking the peptide bonds by acid hydrolysis and passing the amino acids through the column. Once total amino acid N has been determined, a conversion factor can be calculated (Khanizadeh et al., 1992b; Milton and Dintzis, 1981). This factor can be used for subsequent protein determination in tandem with the Kjeldahl method (Khanizadeh et al., 1992b). However, even the use of conversion factors based on total amino acid N analysis still gives an erroneous protein content estimate when used with Kjeldahl total N analysis because of the variation of nonprotein nitrogenous compounds during growth and development (Khanizadeh et al., 1992b).

While the Kjeldahl method is recognized as accurate and precise for total N determination, its application for protein assay in plant materials has been questioned by many investigators (Benedict, 1987; Heidelbaugh, 1975; Jones, 1991; Jones et al., 1991; Khanizadeh et al., 1992b; Milton and Dintzis, 1981).

In summary, because of the small amount of protein present in most plant materials and the existence of many interfering substances, amino acid analysis is the best technique for precise determination of plant protein. This method seems the most reliable for plant tissues and is independent of the type of protein or tissue. It can be used for fresh or dried materials with very low protein content (Horstmann, 1979; Khanizadeh et al., 1989; Marks et al., 1985; Moore and Stein, 1948; Spies, 1957). It is suitable for soluble (i.e., free amino acids, oligopeptides, amino sugars) and insoluble proteins (i.e., cell wall glycoproteins) that are present in large amounts in all plant tissue (Heidelbaugh et al., 1975;

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Horstmann, 1979; Khanizadeh et al., 1989). An added advantage of amino acid analysis using the ninhydrin reaction is the lack of interference by phenolics, tannins, and lignins, which are universally present in plants, often in high concentrations (Khanizadeh et al., 1989, 1992b; Lommis, 1974; Marks et al., 1985). Furthermore, free amino acid content can be measured using the unhydrolyzed methanol extracts of plant samples (Marks et al., 1985). In short, Kjeldahl is not an accurate method for estimating protein content in plant tissue, no matter what the conversion factor is, while amino acid analysis provides a direct measurement of protein in plant tissue rather than an estimation from total N values.

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