Progress in developing a system for direct and simple measurement of protein content in cassava roots

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Introduction

One of the nutritional limitations of cassava roots is their reduced protein content. This affects negatively the diet of millions of people that rely of cassava as staple food. It also affects negatively the price of cassava when used for the composition of animal feed. CIAT, the Institute of Tropical Agriculture (IITA), EMBRAPA (Brazil) and national research programs of many countries have been interested in increasing the levels of protein content in cassava roots. The standard methodology for estimating protein content has been traditionally through the indirect method of quantifying N by the Kjeldahl method and then multiplying the resulting figure by a 6.25 constant. However it has been suggested that the N-to-protein conversion factor, in the case of cassava roots, may be considerably lower than 6.25 because of the presence of non-protein sources of N[1].

Materials and methods

The cassava breeding project at CIAT identified several genotypes that were found to have high levels of proteins (actually, high levels of N)[2]. Crosses have been made among these genotypes. The resulting 393 genotypes were grown in Palmira (Colombia), and flour from their roots obtained. A direct method for the quantification of total soluble proteins contents (TSPC) was tested using the Bradford protein assay, which is a colorimetric method based on the BioRad Dye Reagent (Figure 1). Three aliquots per sample were quantified. A group of 45 genotypes (selected to represent low, intermediate and high levels of TSPC) were also analyzed for their N content using the conventional Kjeldahl method.

Results

Preliminary results confirmed a large variation in soluble proteins with a ten-fold difference between the high and low values (Table 1). However, the actual range of variation for TSPC was "compressed" compared with the variation based on indirect protein quantifications based on N contents. Figure 1 illustrates the relationship between TSPC and protein content based on the Kjeldahl's Method for the 45 genotypes in which N was quantified. This would suggest that the N to protein conversion factor is considerably lower that the conventional factor of 6.25 therefore confirming reports in the literature[1]. The relationship between the two quantification methods was not very strong. The use of protease inhibitors in several samples resulted in similar TSPC suggesting that proteases activity was not relevant in this type of assay.

Table 1. Variation in TSPC in a sample of 393 genotypes using the colorimetric Bradford protein assay. Three aliquots were taken per sample. Coefficient of variation for each individual sample was obtained and is presented in the right column. Reliability of the method is excellent with average coefficient of variation below 6%.

Parameter	Soluble Protein (%)	Coefficient of Variation
Average	0.616144	5.848925
Max	1.02581	32.2906
Min	0.097302	0.102535
St Dev	0.132802	

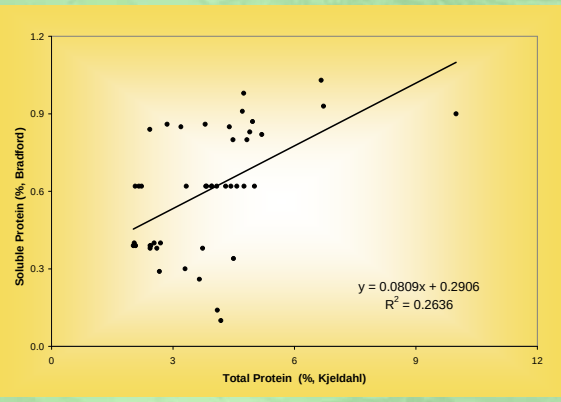

Figure 1. Relationship between TSPC (Bradford method) and crude protein content based on indirect quantification of N content (Kjeldahl's Method) based on 45 samples.

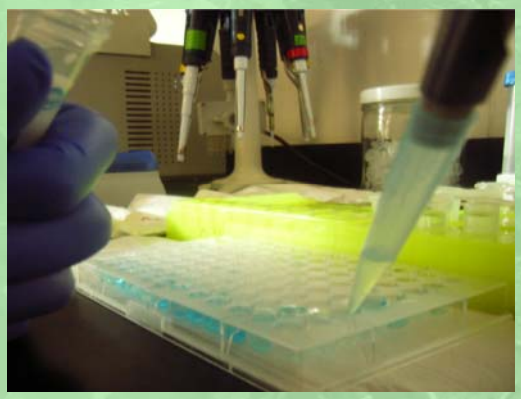

Figure 2. Illustration of the Bradford methodology for quantifying total soluble proteins.

References

[1] Hock-Hin Y, & Van-Den, T. 1996. Protein contents, amino acid compositions and nitrogen-to protein conversion factors for cassava roots. J. Sci. Food Agric. 70:51-54.

[2] Ceballos H, Sánchez T, Chávez AL, Iglesias C, Debouck D, Mafla G, Tohme J. 2006. Variation in crude protein content in cassava (*Manihot esculenta* Crantz) roots. Journal of Food Composition and Analysis 19:589-593