A NOTE ON A MODIFIED TECHNIQUE FOR THE RAPID DETERMINATION OF ZEIN CONTENT IN MAIZE[1]

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A rapid, reproducible method for the determination of the maize prolamine, zein, has been reported (1). The method involves hot-ethanol extraction of powdered endosperm or whole kernels, deposition onto filter paper discs, removal of nonzein impurities by passing the discs through a series of washings, and, finally, micro-Kjeldahl (2) nitrogen analysis of the discs.

The need to run large numbers of micro-Kjeldahl analyses places a physical limitation on the number of samples which may reasonably be analyzed for zein in a given time. To overcome this problem, a procedure has been devised for re-extracting zein from the discs with alkali and performing a colorimetric protein determination on the extract.

Initial attempts to recover zein from the discs quantitatively were unsuccessful. Numerous solvents in which native zein is soluble were tested. Thus, 70% ethanol at 60°C as used in the original step for zein extraction was capable of

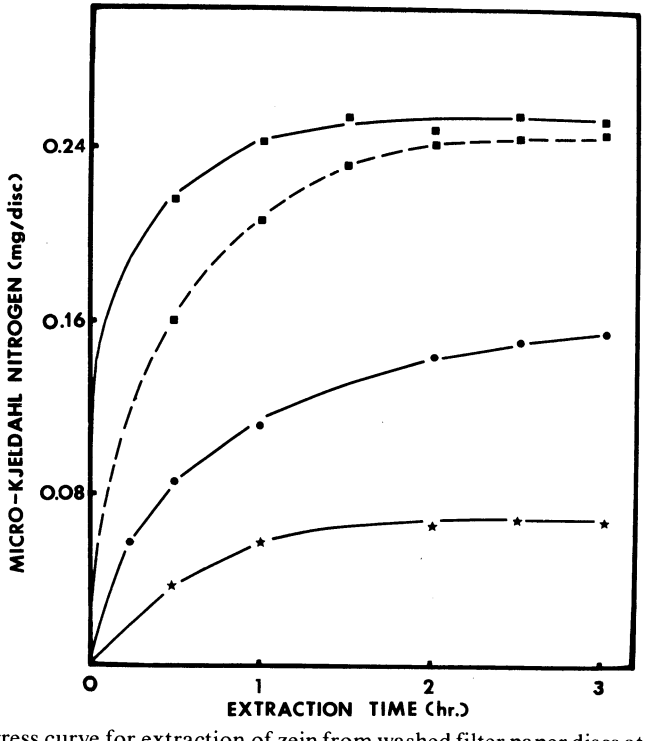

Fig. 1. Progress curve for extraction of zein from washed filter paper discs at 25° C (- - - -) and 60° C (_____) by the solvents 0.1 *N* NaOH (■), 70% ethanol (•), and 0.1 *N* NaOH plus 2% Na_2CO_3 (solid star).

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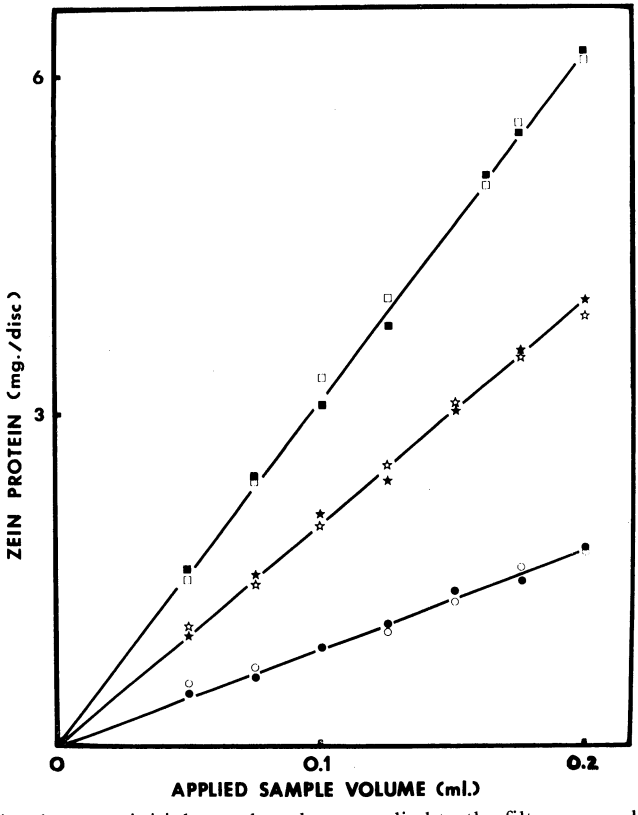

Fig. 2. Relation between initial sample volume applied to the filter paper discs and zein protein recovered by micro-Kjeldahl (open) and colorimetric (closed) analyses. W64A+ (□), W64Ao_2 (O), and W64Afl_2 (open star).

resolubilizing only 60% of the zein applied to the discs (Fig. 1). The use of 1*N* NaOH, as suggested by Lowry *et al.* (3) for the dissolution of insoluble proteins, leads to rapid disintegration of the discs, whereas reagent C ($NaOH-Na_2CO_3$) of the method of Lowry *et al.* dissolves only part of the zein (Fig. 1).

The procedure finally adopted is based on the typical zein extraction curves shown in Fig. 1, using 0.1*N* NaOH as a solvent. Discs (1) are placed individually in 100 × 16-mm test tubes with 3 ml of 0.1*N* NaOH and shaken gently for 2.5 hr at 25° C or 1.5 hr at 60° C. An aliquot of the extract is then analyzed for protein by the colorimetric method of Lowry *et al.* (3).

A comparison between direct micro-Kjeldahl determinations on the discs and colorimetric determination of disc zein from the normal inbred W64A and its isogenic *opaque*-2 and *floury*-2 mutants indicates that good agreement exists between the two methods at all protein levels tested (Fig. 2). Similarly, the modified procedure gives zein values for several normal and mutant versions of maize inbreds which do not differ significantly from zein values based on micro-Kjeldahl analysis (Table I). The determinations reported here agree well with previously published values (1). Following extraction, discs were rinsed with

TABLE I
Comparison of the Micro-Kjeldahl and Colorimetric Methods for Determining the Kernel Zein Content of Normal and Mutant Versions of Several Maize Inbreds

Maize Sample	Zein Content[a] (mg/100 mg sample) ± s.e.	
	micro-Kjeldahl	Colorimetric
W64A+	6.23 ± 0.31	6.10 ± 0.28
W64A*o*$_2$	1.75 ± 0.13	1.97 ± 0.09
W64A*fl*$_2$	3.67 ± 0.28	3.52 ± 0.12
W22+	4.61 ± 0.10	4.37 ± 0.07
W22*o*$_2$	1.54 ± 0.09	1.48 ± 0.06
W22*o*$_7$	1.27 ± 0.10	1.00 ± 0.14
B14+	4.53 ± 0.15	4.72 ± 0.29
B14*o*$_2$	1.35 ± 0.07	1.10 ± 0.07
B37+	4.99 ± 0.05	4.81 ± 0.30
B37*o*$_2$	0.90 ± 0.07	1.01 ± 0.07

[a]Mean of three to six replicates. Single discs from duplicates of each sample were analyzed for protein by each of the two methods. Within each row, means were not significantly different at the 5% level.

TABLE II
Statistical Correlation between Three Methods of Zein Determination

Method[a]	Sample Size (N)	Correlation Coefficient	Significance at 5% Level
HCl *vs.* A_{280}	15	−0.40	none
A_{280} *vs.* A_{750}	15	−0.23	none
A_{750} *vs.* HCl	15	0.65	yes

[a]Protein content determined by: A_{280}, spectrophotometric; HCl, micro-Kjeldahl; or A_{750}, colorimetric method. Zein from B37*o*$_2$ kernels was extracted and purified on filter paper discs. Aliquots of the resolubilized zein (0.10*N* NaOH, 25°C) were then analyzed by the above methods.

base, air-dried, and analyzed for nitrogen by the micro-Kjeldahl method. Significant amounts of nitrogen were not detected. For comparison of the colorimetric and micro-Kjeldahl determined zein values, it has been assumed that the conversion factor of 6.25 (converting micro-Kjeldahl nitrogen to protein) is appropriate. The agreement of zein values determined by the two methods validates this assumption.

The possibility of determining protein content spectrophotometrically at 280 nm on the alkali-extracted disc-zein is precluded by the lack of correlation between zein content determined by the Lowry *et al.* (3) or micro-Kjeldahl methods and that determined spectrophotometrically at 280 nm (4) (Table II). This result suggests the presence of 280 nm absorbing nonprotein contaminants.

The original method of zein extraction and purification (1) coupled with a colorimetric assay may now be utilized for the quantification of zein in maize. This allows those laboratories concerned with assessing the patterns of storage protein metabolism an inexpensive method for the rapid determination of large numbers of samples.

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