

## A Note on Trichloroacetic Acid Precipitation of Oilseed Proteins

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Trichloroacetic acid (TCA) is one of the commonly used protein precipitants. The recommended concentrations of TCA to deproteinize solutions are from 3 to 4% (1), although higher concentrations of TCA have been used to separate protein and nonprotein fractions in extracts of oilseeds (2,3). During studies on the determination of protein nitrogen (N) in extracts of rapeseed, it was found that higher concentrations of TCA than those generally recommended precipitated more proteins from the extracts. Therefore, the effect of TCA concentrations on the precipitation of proteins from extract of three oilseed meals and from solutions of commercial hemoglobin (Hb) and casein was investigated.

### MATERIALS AND METHODS

The oilseeds, rapeseed (*B. napus* L.), sunflower (*Helianthus annuus*), and soybean (*Glycine max*) were defatted with petroleum ether (Skellysolve F, b.p. 30° to 60°C.). Each meal sample (3 g.) was extracted with 120 ml. of 0.2% NaOH for 1 hr. by shaking, and the extracts were centrifuged at 10,000 × g for 10 min. to remove insoluble materials. The supernatant was made to original extract volume with distilled water. Hemoglobin (1 g.) or casein (2 g.) was dissolved in 100 ml. of the

TABLE I. EFFECT OF DIFFERENT CONCENTRATIONS OF TCA ON PRECIPITATION OF PROTEINS FROM EXTRACTS OF THREE OILSEED MEALS AND FROM HEMOGLOBIN (Hb) AND CASEIN SOLUTIONS

		Rape	Soybean	Sunflower	Hb	Casein
Meal N, %		6.5	8.1	8.3	...	...
Alkali-soluble N, mg. <sup>a</sup>		11.5	16.6	22.8	10.4	19.6
% alkali-soluble N precipitated						
	by					
	2.5% TCA	59.8	89.9	87.0	93.7	96.0
	5.0% TCA	68.6	92.6	91.0	93.9	95.8
	10.0% TCA	86.7	93.4	91.6	96.6	96.8
	15.0% TCA	88.0	94.0	92.8	96.2	97.0

<sup>a</sup>In 15-ml. extract or solution.

alkaline solution. An aliquot (1 ml.) of each solution was taken for the determination of total N (alkali-soluble N) by the micro-Kjeldahl method (4). To another 15-ml. portion was slowly added an equal volume of TCA solution so as to obtain final TCA concentrations of 2.5, 5, 10, and 15%. After a stirring time of 5 min. at room temperature, proteins precipitated were removed by centrifugation. An aliquot (2 ml.) of each supernatant was analyzed for total N (TCA-soluble N). TCA-precipitable N (protein N) was obtained by subtracting TCA-soluble N from the alkali-soluble N.

### RESULTS AND DISCUSSION

Table I shows the quantities of proteins precipitated by different concentrations of TCA from alkaline solutions of the three meals and of the Hb and casein. Both Hb and casein, which contain little or no nonprotein N, were almost completely precipitated by 2.5% TCA, even though the molecular weight of Hb is five times smaller than that of the casein. A sixfold increase in the TCA concentration precipitated only 1% more protein from the casein solution and 2.5% more protein from the Hb solution. However, to obtain maximum precipitation of protein (87 to 88%) from extracts of rapeseed meal, TCA concentrations of 10 or 15% were required. A TCA concentration of 5% precipitated 9% more protein, and a TCA concentration of 10 to 15% precipitated 27 to 28% more protein from rapeseed extracts than the protein precipitated by 2.5% TCA. The rest of the N in the extract was nonprotein N not precipitable by the TCA. In the other oilseeds, 5% TCA was sufficient to precipitate most of the protein (91 to 93%) and protein recoveries were only slightly increased by higher concentrations of the precipitant.

The results reported here show that higher concentrations of TCA were required to precipitate rapeseed-meal proteins than to precipitate soybean- and sunflower-meal proteins or casein and Hb. This was probably due to the number and size of the proteins present in rapeseed meal. Both soybean and sunflower meals have been reported to contain four proteins having sedimentation coefficients close to 2, 7, 11, and 15 to 18 S (5,6). These fractions are heterogenous, and in case of soybean have been reported to contain a number of components (6). Wolf has reported (6) that over 80% of the soybean proteins have molecular weights of 100,000 or greater. Rapeseed meal, on the other hand, contains 10 to 12 and

probably more proteins; the largest of these has a sedimentation coefficient of 12 S (7). This protein forms 25 to 30% of the meal proteins. Most of the other rapeseed-meal proteins are smaller species having molecular weights probably in the range of 10,000 to 25,000. Because of their relatively low molecular weights, these proteins precipitate at higher concentrations of TCA.

These data also suggest that rapeseed meal contains more TCA-soluble N than soybean or sunflower meals.

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