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Preparation of Gliadin and Zein.*

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In the course of many years of experience in the preparation of gliadin and zein of a quality suitable for nutrition investigations, convenient and relatively simple methods to obtain these proteins have been devised. So many requests for these methods have been received that publication seems desirable.

Gliadin from Wheat Gluten Flour. Approximately 1 kilo of wheat gluten flour is sifted with vigorous stirring into 7 liters of 70% alcohol at 55-60°C. The mixture is allowed to digest for about 30 minutes and is then poured through a fairly fine cotton gauze (cheesecloth) supported on a flat wire-mesh rack of sufficient area so that not too thick a layer of hydrated gluten is formed and the alcohol runs through freely. After being allowed to drain, the gluten is extracted twice more with 5 liter lots of hot 70% alcohol. The residue in convenient portions is then folded tightly into square cakes in light canvas or drilling press cloth. These are pressed, two or more at a time, between steel plates in the hydraulic press.

The extracts are combined and filtered through a dense pad of paper pulp (4-5 cm. thick) on a large Buchner funnel. The pad is made with an aqueous suspension of pulp and is finally washed with diluted alcohol. The filtrate should be perfectly clear. This filtrate is concentrated *in vacuo* until frothing can no longer be controlled; the addition of an occasional few drops of octyl alcohol is of material assistance toward the end of this operation.

The clear concentrated alcoholic solution is placed in lots of about 1 liter in a large enamelware tub, and water is violently squirted into the tub in such a manner as to cause the greatest possible agitation. Under these conditions, the gliadin separates from solution in the form of a highly aerated froth. This is skimmed off, and, when all has been collected, is thoroughly beaten or whipped with a wire cream beater until homogeneous. The froth is then placed in a thin layer (3-5 cm.) on pans and is rapidly dried in a current of warm air (70-80°C.). After the froth has become somewhat dry on top, any water that has accumulated beneath it is poured off.

* The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington.

The drying must be rapid in order to avoid a collapse of the froth; when correctly dried the product is a crisp, white, much expanded mass. It is crushed by hand and passed through a mill to reduce it to a light somewhat scaly powder.

Gliadin has been prepared in this laboratory by various modifications of this procedure for the past 14 years. The product contains about 15.5% of nitrogen calculated ash- and moisture-free, and is entirely suitable for nutrition studies in which such phenomena as the stunting of growth due to lysine deficiency are to be demonstrated.

Zein from Corn Gluten. Five kilos of dry and finely ground corn gluten (preferably from white corn) are stirred into 20 liters of hot 80% alcohol (50-60°C.) and the mixture is kept hot for about 30 minutes. Filter paper clippings are then added with stirring until a mass is produced that is stiff and dry enough to be molded into cakes which can be folded in canvas press cloth and pressed. The extract obtained from the press is highly colored and quite viscous. It is filtered through a thick pad of paper pulp made on a Buchner funnel as already described. Filtration is slow, but a perfectly clear filtrate is obtained.

The filtrate is treated in convenient portions with an equal volume of ether, and the precipitated protein is thoroughly stirred with the fluid which is finally poured off. This step removes most of the fat and pigment and is less troublesome than the extraction with ethylene dichloride advocated by Mason and Palmer.¹ Reprecipitation of an alcoholic solution of the protein with ether may be carried out if a product of high purity is sought.

The precipitated protein is dissolved in a small amount of warm 80% alcohol and should form a perfectly clear, although very thick and viscous solution. This is transferred to a separatory funnel with the aid of a little diluted alcohol, and the funnel is set up over the following device. A large sheet of cotton gauze (cheesecloth) is spread on a wire-mesh rack in a sink and on it is placed a shallow round pan (milk-pan). Water from a hose which has been fitted with a glass nozzle constricted so as to deliver a rapid flat stream about 2 cm. wide is directed into this pan. A *very thin continuous* stream of the zein solution is then allowed to fall from the separatory funnel upon this stream of water. The zein is partially precipitated at contact and the process is completed in the violently agitated water in the pan. Most of the protein is washed out of the pan but is retained by the gauze. At the end of the operation it

¹ Mason, I. D., and Palmer, L. S., *J. Biol. Chem.*, 1934, **107**, 131.

is collected in the gauze, thoroughly washed with water and is finally allowed to remain in water over night. It is then filtered on a Buchner funnel, spread in a thin layer on pans and allowed to dry at room temperature. Before it becomes thoroughly dry, there is a point at which it can be easily rubbed through a fine sieve whereby a uniform product is obtained. After being thoroughly dried in the air, it is ground to powder in a mill. Such preparations contain approximately 16.2% of nitrogen, ash- and moisture-free, and have been used with success in experiments to demonstrate the peculiar nutritive deficiencies of this protein.

Summary. Methods are described for the preparation of gliadin from wheat and of zein from corn (maize). The products are of a grade suitable for many types of nutrition investigations.

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Blood Chemistry of the Chick Embryo During Ontogenesis.

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Within the last few years increasing attention has been given to studies concerning the changes in the concentration of a number of blood constituents during prenatal development. With the aid of such data it may be possible to add further information concerning the utilization of the various foodstuffs during the embryonic period. Further, it may be possible, by correlating the extent of functional development of the various organs of the embryo at a given age with such chemical blood data, to throw further light upon the influence of a given organ upon intermediary metabolism.

The chick embryo was chosen as the experimental animal. The eggs were incubated under standard conditions and at the desired age the egg was carefully opened and sufficient blood for analysis was withdrawn from the vitelline artery or vein into a 1 cc. tuberculin syringe. Since satisfactory blood samples could not be obtained from embryos of less than 9 days incubation no analyses were attempted until that time. From this period on, however, the analyses were carried on through the entire incubation period and for several days after hatching.