Proteins in Milk, Grains, and Oilseeds

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Compared with scientific understanding of carbohydrates and fats, relatively little was known 50 years ago about the chemistry of proteins. "Of all natural products," wrote a chemist in Peoria during the early years, "the proteins present the most complicated puzzle with which science has to deal." Since then, of course, the body of knowledge about protein chemistry has been expanded enormously. A significant part of this increased understanding has been acquired through research at the four ARS regional laboratories. Following is a sampling of some of the more important discoveries that have taken place during the last half century in Peoria, Albany, Wyndmoor, and New Orleans.

Milk. In cow's milk, casein is by far the most abundant protein, and it was one of the first studied in depth at the Eastern laboratory. For a long time, casein was thought to be a simple substance, but ERRC scientists soon learned that this was a misconception. Early analytical tools were just too crude to reveal its complexity. For example, "simple" casein was eventually found to contain four component proteins: alpha-, beta-, kappa-, and gamma-casein. Further investigation revealed still greater complexity in alpha-casein, which contains both calcium-sensitive and calcium-insensitive fractions. It is the interaction of these four proteins that creates the colloidal complex that transports calcium, an essential mineral in our diets.

Examination of milk from individual pedigreed cows revealed still more casein types—mutant forms known as genetic variants. ERRC researchers tracked the presence of these variants in different breeds and blood lines, observing their relative abundance, the chemical differences in their molecules, and their relationship to the quality of milk and milk products. This research provided important information in the genetic study of dairy cows and led to practical improvements in milk processing.

Alphabet of Life

Amino acids are sometimes called the alphabet of proteins, sometimes the building blocks. Despite the enormous diversity of protein molecules in living things and their great size (hemoglobin in human blood has a molecular weight of 63,000), all are made up of combinations of 20 standard amino acids. The human body can manufacture 12 of these acids from the raw materials supplied by our diet. The remaining 8, however, must be acquired readymade from the food we eat.

These 8 essential amino acids are valine, lysine, threonine, leucine, isoleucine, tryptophan, phenylalanine, and methionine. The proteins of certain foods, like meat, fish, poultry, eggs, milk, cheese, and a few legumes, contain adequate amounts of all of them and are known as complete proteins. The proteins in other foods—grains, nuts, vegetables, and fruits—are deficient in one or more of the essential acids or contain too little protein overall to meet the body's needs.

Other milk research revealed not one but three types of beta-lactoglobulin, the chief protein in whey after casein has been removed to make cheese. The types are genetically variable. ERRC scientists also developed a practical method for probing the complex molecular structure of proteins, and biochemical research revealed for the first time how casein is modified (phosphorylated) to carry calcium. This was responsible for much of what scientists eventually learned about casein and beta-lactoglobulin. The findings have led to new insights into the use of milk proteins as food ingredients with high nutritional benefits.

Wheat. Like other grains, wheat is a nutritionally incomplete protein, deficient in lysine and threonine, two essential amino acids. Nevertheless, it provides 15 percent of the protein in the U.S. diet. One reason for wheat's popularity is that, thanks to the elasticity of its gluten, it is the only grain besides barley (which doesn't do it nearly as well) and triticale (a cross of wheat and barley) that can be made into a bread dough that rises. Gluten is composed mostly of protein.

Scientists at Peoria began fundamental research on wheat gluten in 1955, seeking to establish a basis for determining wheat quality. Their work resulted in a long list of firsts. They were the first to isolate and characterize wheat gliadins, one of the two protein fractions that make up gluten. They were also the first to isolate subunits of wheat glutenin, the other fraction in gluten. It is the glutenins that contribute most of the strength and viscosity to flour doughs and make wheat flour so useful for bread and other bakery items. This basic information has helped industry to use and control the properties of gluten proteins and of the wheat products that contain these proteins (see "Wheat, Flour, and Bread," p. 109).

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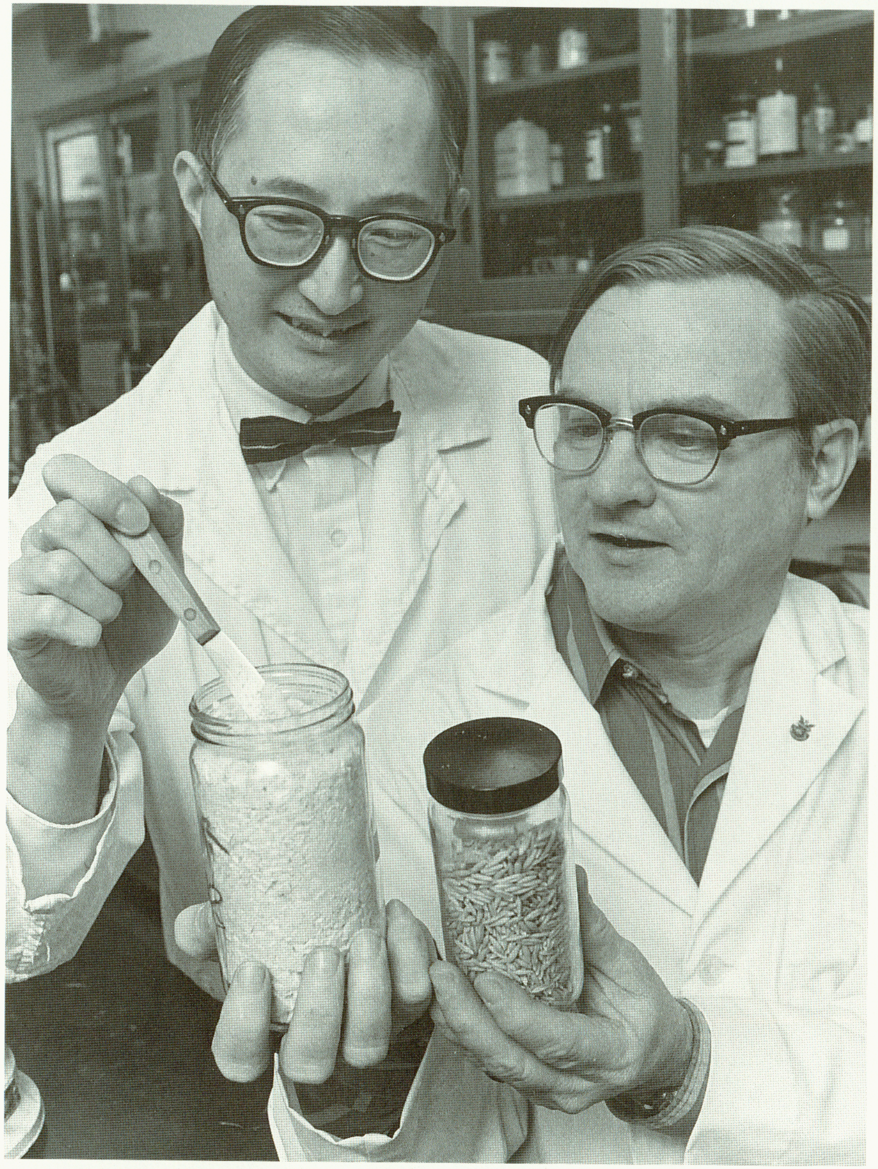
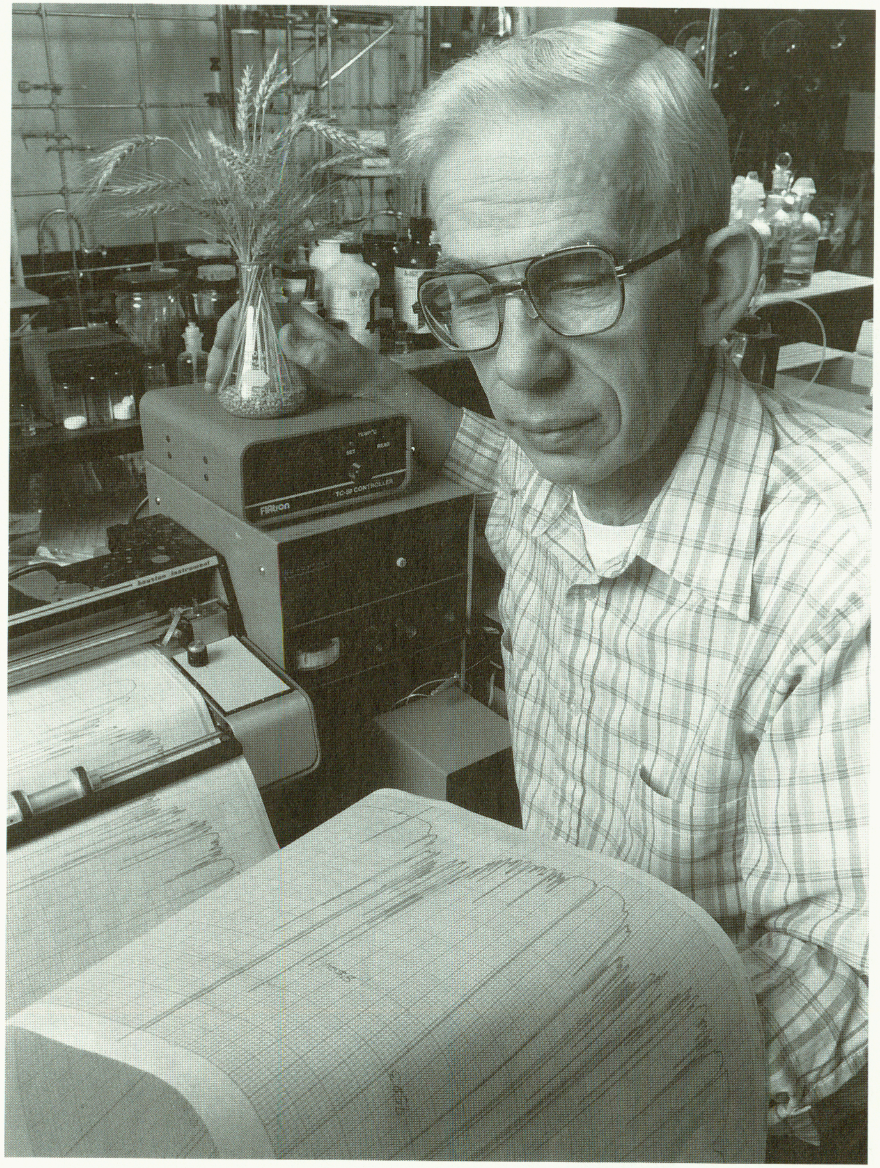
NRRC researchers were also the first to use high-performance liquid chromatography to analyze wheat proteins. A procedure using the same instrumentation was later developed to identify different wheat varieties by analyzing the levels of individual proteins in a single kernel of wheat. The method, which is fast and reproducible, is comparable to fingerprinting and aids the wheat breeder by identifying desirable genetic characteristics. It continues to provide important information for wheat researchers.

But much more remains to be learned about wheat proteins. Learning how gluten fits together and interacts with other food components in bread is one of the greatest challenges confronting protein chemists. One researcher explains that gluten has some 100 to 200 different subunits joined in an "incredible number of combinations" that affect the nutritional and physical qualities of the proteins.

The Western laboratory has also conducted research on wheat proteins, focusing most recently on opening the way to making genetic improvements in the grain. Through gene sequencing and direct protein sequencing, WRRC researchers have learned how gluten proteins determine the unusual elastic properties of doughs—and why there are important differences in these properties among various wheat varieties. In the laboratory at



Oat protein, isolated and concentrated by chemists Y. Victor Wu (left) and James E. Cluskey and their associates, has the best amino acid balance of the cereal proteins.



In Peoria, chemist Floyd Huebner separates proteins of hard red wheat using a high-performance liquid chromatograph.

least, scientists have modified the amino acid content of major proteins in the wheat kernel through gene manipulation. Objectives are to enlarge the natural germplasm base of wheat and to make improvements in the grain that would be practically impossible with traditional breeding methods. Future improvements, of course, will ultimately require the help of plant breeders.

In other WRRC research, a new biotechnology probe comprises antibodies that seek out and bind to wheat glutenins. It may prove a faster, easier way to track and measure these protein fractions.

Corn. While the bulk of each year's corn crop is used directly as feed for farm animals, some 1.2 billion bushels, or about one-sixth of the harvest in a typical crop year, is used for human food here and abroad, to make alcohol, corn sweeteners, and industrial products, and for seed. Corn gluten, a byproduct of cornstarch refineries, contains about 50 percent protein, and much of this product is also used in feed concentrates for animals. NRRC scientists, however, have worked long and hard to develop industrial uses for corn proteins, with varying degrees of success.

Of particular interest to NRRC scientists in the 1940's and 1950's was zein, an alcohol-soluble corn protein first produced commercially in 1938. In 1945, Northern lab researchers developed a complicated method for extracting zein from corn gluten meal. By 1948, industry was using the process to produce zein for many industrial purposes.

For a time, when the corn protein was competitive in price with other protein sources, zein was used with resin and alcohol to make varnish. It was also used as a substitute for shellac in phonograph records, as a binder in composition cork, and for dozens of other uses in making paper and paperboard, gaskets, coatings, and adhesives. In 1948, it was even made into a warm, durable textile fiber—Vicara—which was used in fabrics alone or in blends with rayon, nylon, and wool. After years, however, the zein in many industrial products was replaced by lower priced compounds, including petrochemicals.

WRRC food chemist Linn P. Hansen compares standard-process rice flour, in container on left, with enzyme-treated CHP rice flour that has three times more protein.

Much of the concern of agricultural scientists over corn protein has centered on keeping its content high in feeds and food. In the mid-1950's, an NRRC chemist observed that the protein content of the U.S. corn crop since the mid-1930's had fallen from about 11 percent to an average of less than 10 percent. Further study convinced him that the amount of protein in corn was strongly influenced by climate, and in particular by lower average temperature. His findings led to more intensive study of the impact of weather and hybridization on corn nutrients and were of great value to breeders.

Within several years, breeders had developed new varieties of corn with higher protein content, but the new lines were often too soft for efficient milling. Analytical data from NRRC researchers, much of it obtained through the use of sophisticated techniques and instruments, led to the development of new lines with good nutritional properties and harder kernels. These higher protein corns have proved valuable for many uses, including inclusion in CSM, a corn-soy-milk blend exported for the Food for Peace Program (see "Food for Peace," p. 116).

In 1973, NRRC cereal chemists developed dry-milled corn germ flour, a new product. It was a significant new source of iron and other minerals and of protein, including the amino acids lysine and tryptophan. Taste panels agreed that in cookies and similar baked goods, corn germ flour could replace 25 percent of less nutritious wheat flour with no discernible change in taste or texture of the products. In 1976, one company began making and marketing the flour, but after the death of the firm's owner, it was no longer produced in this country. Currently, there is interest in food products containing protein recovered from corn stillage after starch is converted to alcohol.

Soybeans. As early as the 1940's, it was evident that oil-free meal from dehulled soybeans, a produce with about 50 percent protein, is a raw material with several important industrial applications. By 1950, it was used to make as much as 45 million pounds of waterproof soybean meal glue for fabricating plywood. It also found a market in coating wallpapers, and later found use as a glossy coating for top-quality printing papers.

The first important use of soybean protein in human foods came in 1950 with the invention at the Northern lab of an edible high-protein product, the result of extracting oil from soybeans with ethanol, or ethyl alcohol. The alcohol extraction method improved the color and taste of the meal and of the protein isolated from it. The new NRRC product, which was made from alcohol-washed defatted soybean flakes, was named Gelsoy; it was 55 percent protein and 45 percent soluble carbohydrate. In solution with water, it could be whipped into a meringue like egg white or used in ice cream as a vegetable gel. Within a few years, it was discovered that Gelsoy could be used to impart more softness to bread, a quality prized by many consumers. The product was soon being produced by a Peoria miller.

A short time later, the hexane-and-alcohol method for extracting soybean oil led to development of several products produced commercially: a soy flour, a protein concentrate, and a textured protein concentrate. The flavor of these products was so improved over early examples of soy flours and extenders that by 1979, all ground beef purchased by the U.S. military was extended 20 percent with textured soy protein. The products were used in schools, nursing homes, and institutions, and proved popular as a source of protein for vegetarians.

A new type of soy concentrate was isolated from full-fat soy flour in the mid-1970's by a chemical engineer in Peoria. The concentrate could be reconstituted easily with water to make a bland, smooth soy "milk" for feeding babies allergic to cow's milk. It could also be used in other beverages, including thick milkshakes. The concentrate retained 94 percent of the protein and 85 percent of the oil in soybeans, and cooking the soy inactivated enzymes that would limit shelf life.

At the Southern center, researchers have modified soy proteins with enzymes to improve the solubility of soy flour and its ability to form emulsions. The enzyme treatment, which is now being evaluated by industry, may extend the use of soy flour in coffee whitener, salad dressings, and protein-fortified beverages.

Meanwhile, soybean researchers at several labs, including the NRRC, have been zeroing in on the plant's production of oil and protein. By 1991, three soybean genes had been cloned that are

Proteins

Protein is an essential constituent of all living cells, plant and animal. The human body is about 18 percent protein. It is needed to build body tissues and to construct hormones, enzymes, and genetic materials. Protein is one of the most complex of all organic substances.

Proteins are composed almost entirely of the elements carbon, hydrogen, oxygen, and sulfur. Some contain small amounts of phosphorus and other elements. Most plants make their own protein from photosynthetic products and inorganic nitrogen from the soil. Animals, including people, have to get their raw materials for building proteins—the amino acids—either by eating plants or other animals.

The number of different proteins in plants and animals is astronomical. There are 3,000 in a common bacterium, *E. coli*; there may be 100,000 in a human being. But the proteins in each kind of living thing—a house fly, a dandelion, a goat—are unique to that species, although there are amazing similarities in closely related species.

Proteins in agricultural products include albumin in eggs, gluten in cereals, casein in milk, keratin in feathers, collagen in hides, and other proteins in the various oilseeds. Essential to our diet, proteins are also used for a variety of industrial purposes.

important to the production of hitherto scarce amino acids essential to human nutrition. Today, soy protein products, including infant formulas, make a moderate-sized industry.

Cottonseed. Researchers at the Southern center in the 1980's developed a simple, air-separation process for making an edible, 65-percent-protein flour from cottonseed. It reportedly has chemical and physical properties that make it attractive for use in food products. It also meets standards for free-gossypol content of the Food and Drug Administration and the UN's Protein Advisory Group. SRRC engineers say that the flour's price in most years would be competitive with the price of soybean protein concentrate.

Rice. Scientists at both the Southern and Western labs have developed high-protein rice flours. The SRRC uses an overmilling procedure to remove the protein-rich outer surface of the rice kernel for use in its product. The WRRC uses an enzyme obtained from a mold, *Aspergillus oryzae,* to produce CHP (chemically high protein) rice flour from standard rice flour.

Oats. Chemists at the Northern lab isolated and concentrated oat protein in the mid-1970's. The product has a bland taste, good nutritional value, and can be produced in commercial quantities. Researchers foresee using oat protein in meat products and in baked goods, where it can hold moisture to help maintain freshness.

Safflower. At the Western lab, scientists invented a new process for extracting protein from safflower, an oilseed plant that is tolerant of drought. In many applications, the protein is said to exceed the standards set by users of soy-based protein. The safflower protein isolates are suitable for enriching pastas and carbonated beverages. Spin-off research was under way in 1991 to make use of the protein to fortify tortillas made from corn or wheat flour.