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FACTORS FOR CONVERTING PERCENTAGES OF NITROGEN IN FOODS AND FEEDS INTO PERCENTAGES OF PROTEINS

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INTRODUCTION

The importance of proteins in the diet is so generally recognized as to need here no detailed discussion. Both the quantity and the quality of protein are matters of importance to everyone, particularly to dietitians, physicians, housewives, school and institutional workers, food and feed manufacturers, and animal husbandrymen. Unless the diet contains enough protein of satisfactory quality proper nutrition will not result. An excess of protein, on the other hand, is at least uneconomical, because protein is the most costly of the food elements. The percentage of protein in a feed largely determines its price. Wheat is now commonly bought on the basis of its protein content. It is, therefore, desirable to have as accurate knowledge as possible of the quantity of protein in various materials.

Proteins have been defined as nitrogenous chemical compounds of great molecular weight which give colloidal solutions and which on analysis yield amino acids as cleavage products.

Without proteins there can be no life. They play a predominating rôle in the life process of every cell. Proteins enter into the construction of nearly all animal tissues and form the chief constituent of many of the body fluids. Aside from their rôle in nutrition, they are connected with many physiological processes in health and disease. Serums, toxins, antitoxins, sensitization, immunization, allergy—terms familiar in the modern practice of medicine—are largely interpreted in terms of protein.

Fat, carbohydrate, ash, and moisture in food materials can be determined with a high degree of accuracy, but the method used for estimating protein is unsatisfactory. The percentages for protein given in books and tables showing the composition of foods are merely a form of expressing the total nitrogen, and in many cases are far from representing the correct values for real protein.

Investigations on proteins carried on in the Bureau of Agricultural Chemistry and Engineering and elsewhere during recent years have yielded information regarding the nature and composition of the proteins in a large number of different food materials. It is the purpose of this circular, in the light of these results, to present some data which will contribute to a more accurate estimation of the protein content of various foods and feedstuffs. Also some of the shortcomings and limitations of our present method of estimating protein are pointed out and discussed, so that those using the percentages given for protein in various materials can do so more intelligently by making certain allowances.

The new protein conversion factors presented in this circular are based upon the most reliable information available regarding the nature and composition of the proteins in the materials concerned. Although it is realized that their use will not give values which will express the quantity of protein in the different food materials with absolute accuracy, it is believed that they will give values representing the real protein content more closely than those obtained by the indiscriminate application of the factor 6.25, now in general use. How these factors are to be applied must be left to the discretion of those who wish to use them in their own particular fields.

QUALITY OF PROTEINS AS A FACTOR IN NUTRITION

One of the outstanding discoveries made within a comparatively recent time in the field of nutrition is that proteins differ not only with respect to their chemical make-up, but also in their food value.

Progress in the field of nutrition has necessitated a revision of the ideas which formerly prevailed regarding the nutritional requirements of an animal. Formerly all foods were classified according to four basal factors—proteins, fats, carbohydrates, and mineral constituents. A diet was considered complete which contained these four factors in sufficient quantities to meet the physiological needs of the animal. In the older appraisement of food values, little consideration, if any, was given to the quality of the protein. A protein was a protein, and its food value was largely judged by the number of calories it would furnish. Today we know that a diet may supply an abundance of calories, and may contain a sufficient quantity of protein, fat, carbohydrates, inorganic salts, and vitamins, and yet be inadequate to provide for the normal growth and maintenance of an animal if the protein is not of the right quality.

The quality of a protein depends on its content of certain compounds called amino acids, of which proteins are primarily composed. Most proteins contain some 18 different amino acids, 4 of which, at least, have been amply demonstrated to be essential for the satisfactory growth and nutrition of animals. These are lysine, tryptophane, histidine, and cystine. These amino acids must be supplied by the food that is eaten, as the body can not manufacture them from other substances.

QUANTITY OF PROTEINS IN FOODS AND FEEDS

Research on the proteins of food materials resolves itself, therefore, when viewed from a practical standpoint, into a study of the quantity and quality of the different proteins in foods and feedstuffs.

During the last 15 years the Protein and Nutrition Research Division of the Bureau of Agricultural Chemistry and Engineering has carried on extensive investigations on the proteins of different materials and their constituent units, the amino acids.[1] As a result, many new data have been accumulated relating to the proteins of many foods. The proteins of some 51 different food materials have been isolated in as pure condition as possible, and their properties and amino acid composition have been determined. Several have been obtained in crystalline form.

Both in human nutrition and in the practical feeding of farm animals it is obviously a matter of great importance to have as accurate data as possible, not only regarding the quality of the different food proteins but also regarding the quantity in which they are present in the various foods. For the feed manufacturer and the practical feeder of farm animals it is an ever-present problem to know how much of the different ingredients to use when compounding a ration so that the mixture will contain, for example, 18 or 20 percent of protein of good quality. The same question confronts the dietitian. A diet containing a definite percentage of protein is prescribed for a patient. What proportions of the different foods that are to constitute this diet must be taken so that the diet will contain the definite quantity of protein required?

Again, in scientific experimental work diets and rations are frequently required that contain a definite, known percentage of protein.

ESTIMATION OF PROTEIN IN FOODS AND FEEDS

In order to produce a mixture of several ingredients that will contain a given quantity of protein from known sources, it is obviously necessary to know the percentage of protein in each of the several ingredients. This is ascertained by referring to tables which give the percentages of protein, fat, and carbohydrate in different food materials. The percentages of protein as there given are then used in calculating the quantity of each constituent necessary in order that the mixture shall contain the required quantity of protein.

Many users of food-composition tables will doubtless be somewhat surprised to learn that the percentages given for protein in different foods represent the nitrogen content, and in only a few cases do they

[1] The results of these investigations are described in some 73 articles which have been published in various scientific and technical journals. The supply of reprints of most of these articles is exhausted, but the original articles can be consulted in the publications cited in the bibliography at the end of this bulletin.

represent the actual protein. In many cases the percentages given for protein are far from correct, and are likely to be misleading. Much has been detracted from the practical and scientific value of many experiments in which the quantity of protein in the ration or diet has been calculated only on the basis of nitrogen content.

In biological tests, unless pure, isolated protein is being used, or the effect of nonprotein nitrogen is eliminated, would it not be more accurate to speak of the protein value of corn, for instance, rather than the biological value of its protein?

The quantity of protein in materials can not be determined with expediency by separating and weighing the pure protein as is done with fats and other food constituents. The great difficulty and time-consuming labor involved in the quantitative isolation of proteins in a pure condition absolutely preclude this method. Instead, the percentage of protein in a material is calculated from the quantity of nitrogen it contains. The nitrogen is usually determined by the Kjeldahl method or some modification of it.

All proteins contain nitrogen. Many years ago, when the comparatively few proteins known were chiefly of animal origin, such as serum albumin and serum globulin from blood and casein from milk, it was found that these proteins contained about 16 percent nitrogen. On the assumption, therefore, that proteins in general contained 16 percent nitrogen, the practice originated of estimating the percentage of protein in nitrogenous food materials by multiplying the percentage of nitrogen by the factor 6.25 ($100 \div 16$). With but few exceptions, the percentages of protein in foods as given in food-composition tables have been calculated in this way; that is, by multiplying the percentage of nitrogen by the factor 6.25.

This method of estimating protein rests on two assumptions, neither of which is necessarily correct. In the first place it assumes that all the nitrogen in food material is protein nitrogen. It is known, however, that in many, if not in most cases, nitrogenous substances are present which are not proteins nor related to proteins. In the second place it assumes that all proteins contain 16 percent nitrogen.

NONPROTEIN NITROGEN IN FOODS

In connection with the first assumption referred to, it is obvious that an estimation of the percentage of protein based on the nitrogen content of a material can not be accurate if that material contains nitrogenous constituents other than protein. There are few, if any, naturally occurring nitrogenous substances that do not have constituents other than protein which also contain nitrogen. An estimation of protein based on total nitrogen content will, therefore, give values that are too high. The following two cases may be taken as illustrations. Only one-third to one-half the nitrogen of the common potato has been accounted for as protein. Concerning the nature of the remaining nitrogen in the potato but little is known. It has been shown, however, that a considerable proportion of this nonprotein nitrogen belongs to asparagine. Potatoes, therefore, may contain not 1.5 to 2 percent of protein (nitrogen$\times$6.25), as given in tables showing their composition, but only one-half, or less, of that amount. Again, alfalfa, an important feedstuff, and one of the very few substances the nature of the total nitrogen of which has been systematically investigated, has been shown to contain a number of nitrogenous compounds which are not even related to protein (*86*, *99*, *100*, *101*, *102*, *103*, *104*).[2] The following are some nonprotein compounds containing nitrogen that have been isolated from alfalfa leaves: Stachydrine, asparagine, choline, adenine, trimethylamine, betaine, arginine, lysine, tyrosine aspartic acid, alanine, valine, leucine, phenylalanine, serine, and glutamic acid. Unquestionably there are other nonprotein compounds in alfalfa that have not yet been discovered. Here again as in the case of potatoes it is evident that the protein percentage calculated on the basis of total nitrogen must be far too high.

The statement is sometimes made that, from a nutritional standpoint, the nonprotein nitrogenous compounds in food materials may have a value quite equal to that of protein itself, and that, for this reason, it is immaterial whether the protein content is based on the total nitrogen or on the actual protein present. This might be true, if amino acids were the only nonprotein compounds involved. But, as is well known, a large number of nitrogenous compounds exist, both in plant and animal material, which can not replace protein in the diet.

It is not the purpose of this discussion to convey the idea that the method of estimating proteins by basing it on the total nitrogen should be abandoned, but to point out some of its limitations so that those using it may be cognizant of its inaccuracies and can be guided accordingly.

At present one great obstacle to progress in the estimation of protein in food material is the lack of information regarding the nonprotein nitrogenous constituents of food material. With one or two exceptions, no plant or animal material has been systematically studied with the object of learning the nature of its total nitrogenous constituents. Most investigators have been content merely to isolate and identify individual compounds. Knowledge of the nature of the total nitrogenous constituents of food material is essential, not only for estimating their protein content but also for ascertaining their food value.

NITROGEN CONTENT OF DIFFERENT PROTEINS

As pointed out in the preceding paragraphs, the error involved in estimating protein upon the basis of total nitrogen unfortunately can not be corrected until much more information is available regarding the nonprotein constituents. With reference, however, to the second source of error in estimating protein, namely, that arising from the indiscriminate use of the conversion factor 6.25, we do have data which can be applied for the calculation of factors that will give results representing more nearly the true protein content. The chief proteins in most of our common food materials have been isolated and studied. The percentage of nitrogen in these proteins is definitely known.

In table 1 is given a list of different proteins that have been isolated from plant and animal sources, and the percentages of nitrogen which these proteins contain. Most of the proteins listed

[2] Italic numbers in parentheses refer to Bibliography, p. 16.

have been prepared and analyzed many times. Many of the nitrogen percentages were determined in the Protein and Nutrition Research Division of the Bureau of Agricultural Chemistry and Engineering. The others have been compiled from the work of various investigators. In nearly all cases the percentage listed in the table for any given protein varies but little from the percentages recorded for other preparations of the same protein, many of which were determined by several investigators.

TABLE 1.—*Nitrogen content of various proteins* [1]

Source of protein	Protein	Nitrogen
		P. ct.
Adzuki beans	α-Globulin	15.6
	β-Globulin	16.5
	Legumelin	16.3
Almond	Amandin	19.3
Alfalfa leaves	Protein	15.8
Avocado	Salt-soluble protein	15.3
	Alcohol-alkali-soluble protein.	13.4
	Alcohol-soluble protein.	16.2
Barley	Hordein	17.2
	Globulin	18.1
	α-Glutelin	16.2
	Albumin	16.6
Blood	Serum globulin	16.2
	Serum albumin	16.0
	Hemoglobin	16.8
	Fibrin	16.9
Brazil nut	Excelsin	18.3
Buckwheat	Globulin	17.4
Butternut	do	18.6
Cantaloupe seed	do	18.4
Castor bean	do	18.8
	Ricin	16.6
Chicken	Muscle fiber	16.1
Cohune nut	Globulin	17.8
Corn	Zein	16.1
	Glutelin	16.1
	Globulin	18.0
Corn leaves	Protein	14.4
Coconut	Globulin	18.5
Cottonseed	α Globulin	18.2
	β-Globulin	17.8
Cowpea	Vignin	17.2
	Legumelin	16.4
Durum wheat	Prolamin	17.5
Eggs	Ovalbumin	15.5
	Conalbumin	16.1
	Vitellin	16.3
	Livetin	15.1
Einkorn	Prolamin	16.7
Emmer	do	17.6
Filbert	Corylin	19.2
Fish (halibut)	Muscle fiber	16.5
Flaxseed	Globulin	18.5
Gelatin	Gelatin	18.0
Hazelnut	Corylin	19.1
Hempseed	Edestin	18.7
Jack bean	Canavalin	16.7
	Concanavalin	16.4
Kafir	Kafirin	16.6
Lentil	Legumin	18.1
	Vicilin	17.4
	Legumelin	16.3
Lima bean	α-Globulin	15.5
	β-Globulin	14.8
	Albumin	14.2
Locust tree bark	do	14.8
Lupine	Conglutin	18.0
Marigold seed	Globulin	16.9
Maple seed	Acerin	18.3
Milk (cow's)	Casein	15.9
	Lactalbumin	15.4
	Alcohol-soluble protein.	15.7
Mung bean	α-Globulin	15.7
	β-Globulin	16.7
Navy bean	Phaseolin	16.1
	Conphaseolin	15.7
Oat	Prolamin	16.4
	Glutelin	17.5
	Globulin	17.9
Ox (flank)	Muscle fiber	16.2
Pea	Legumin	18.0
	Legumelin	16.3
	Vicilin	17.4
Peach kernel	Globulin	19.3
Peanut	Arachin	18.3
	Conarachin	18.2
Potato	Tuberin	16.4
Radish seed	Globulin	18.2
Rapeseed	do	17.0
Rice	Globulin, coagulable at 74° C.	16.3
	Globulin, coagulable at 90° C.	17.9
	Oryzenin	16.8
	Prolamin	16.2
Rye	Gliadin	17.7
	Glutelin	16.7
	Albumin	16.7
Scallop	Muscle fiber	17.0
Sesame seed	α-Globulin	18.4
	β-Globulin	17.6
Shrimp	Muscle fiber	16.9
Silk	Sericin	15.9
	Fibroin	18.0
Soybean	Glycinin	17.5
	Legumelin	16.1
Sorghum	Prolamin	14.3
Spelt	do	17.0
Spinach	Spinacin	16.2
Squash seed	Globulin	18.5
Sunflower seed	do	18.6
Sweetpotato	Ipomoein	16.2
Teosinte	Prolamin	15.9
Tomato seed	α-Globulin	18.3
	β-Globulin	16.0
Velvetbean, Georgia	α-Globulin	16.6
	β-Globulin	17.3
	Albumin	15.9
Velvetbean, Chinese	Stizolobin	16.3
Vetch	Legumin	18.0
	Legumelin	16.2
Wheat endosperm	Gliadin	17.6
	Glutenin	17.5
	α-Glutelin	17.1
	β-Glutelin	16.1
Wheat germ	Leucosin	16.8
	Globulin	18.3
	Proteose	17.0
Wheat bran	Prolamin	15.3
	Globulin	17.7
	Albumin	15.4
Walnut, English	Juglansin	18.9
Walnut, black	do	18.9

[1] The percentages given for the proteins of the various seeds are those for the dry, mature material.

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ERRORS ARISING FROM THE USE OF WRONG CONVERSION FACTORS

As shown in table 1, the nitrogen content of different proteins varies within a rather wide range. Some proteins that have been isolated contain as little as 13 percent nitrogen; others contain more than 19 percent. As an illustration of a possible error in calculating the protein content of a single food material by using the factor 6.25, take the case of almonds. The protein content of the edible portion of almonds is usually given in tables showing the composition of foods as 21 percent. This figure is obtained in the conventional manner by multiplying the nitrogen content of almonds, 3.36 percent, by the factor 6.25. However, the protein of almonds consists of a globulin named amandin, containing 19.3 percent of nitrogen. This protein has been isolated in crystalline form (*90*) and has been extensively studied. No other protein has been found in significant quantities in this seed, and it has but little nonprotein nitrogen. Its nigrogen content, 19.3 percent, corresponds to the factor 5.18. If the factor 5.18 be used, which corresponds to the actual percentage of protein nitrogen in almonds, instead of the conventional factor 6.25, the percentage of protein in almonds will be found to be 17.4 percent instead of 21 percent.

Even more striking is the discrepancy in the case of gelatin. Its protein content is given as 91.4 percent ($N \times 6.25$). However, when the factor 5.55 is used, which is the one corresponding to the actual nitrogen content of gelatin, the protein content will be found to be 81.2 percent. Striking differences in the figures for other food materials are shown in table 6.

The chief proteins in most vegetable material contain more than 16 percent nitrogen. This is particularly true of seed proteins. Many seeds, however, do contain besides the one or two chief proteins, one or more other proteins in smaller quantities, which almost invariably contain less nitrogen. If only those proteins which make up the greater part of the protein content of vegetable material be taken into consideration, the average percentage of nitrogen in them is about 17 percent. This corresponds to the conversion factor 5.84. When the percentages of protein in most foods of vegetable origin, with the possible exception of green vegetables, fruits, and tubers, are being calculated, this factor, therefore, would give the true protein content more closely than the factor 6.25, which is now used. There are certain foodstuffs concerning the proteins of which sufficient is known to justify the use of special nitrogen conversion factors. Although in most cases even these factors will not give absolutely correct figures for protein content, they will give them more accurately than the arbitrary factor 6.25. Even for substances the nitrogen of which is known to be protein nitrogen, and for which the relative proportions of the different proteins have been fairly well established, available data are not sufficiently complete for the calculation of perfectly correct factors.

CHEMICAL COMPOSITION OF PLANT PROTEINS IN RELATION TO THE PLANT ENVIRONMENT

The use of any factor for the conversion of the percentage of nitrogen into terms of protein in a given material presupposes that the nitrogen content of the individual proteins contained in that material is constant. For example, the adoption of the conversion factor 5.7, which is generally used for wheat and wheat flour, is based on the assumption that the nitrogen content of gliadin and glutenin is independent of the environment under which the wheat was grown, such as climatic and soil conditions; also that the gliadin and glutenin obtained from different varieties and classes of wheat contain the same percentage of nitrogen. In other words, it assumes that wheat gliadin and wheat glutenin are chemically definite proteins or groups of proteins that have a constant chemical composition irrespective of the conditions under which the wheat from which they are obtained is grown. This must not be confused with the well-known fact that different classes of wheat and wheat grown in different environments vary with respect to the total quantity of protein they contain and also with respect to their ratio of gliadin to glutenin content. But these two proteins, in whatever quantities or in whatever ratio to each other they occur, have always been found to contain practically the same percentage of nitrogen. This has been demonstrated by the analyses of hundreds of preparations of these proteins by different investigators covering a period of more than 80 years. The remarkably close agreement of the nitrogen content of gliadin and glutenin prepared by different workers not only in different sections of the United States but also in other countries is shown in tables 2 and 3.

TABLE 2.—*Nitrogen content and specific rotation of gliadin prepared from wheat of different varieties and origin as determined by various investigators*

Investigator	Year	Nitrogen	Specific rotation[1] $(\alpha)^{20}_{D}$	Investigator	Year	Nitrogen	Specific rotation[1] $(\alpha)^{20}_{D}$
		P. ct.				*P. ct.*	
Gunsberg (*82*)	1861	17.76		Gröh and Friedl (*81*)	1914	17.53	−91°
Ritthausen (*96*)	1862	17.58		Luers (*87*)	1919	17.20	−91.3°
Kjeldahl (*85*)	1892	17.25	[2] −92°	Woodman (*105*)	1922	----	93.6°– −93.8°
Osborne and Voorhees (*95*)	1893	17.66		Eto (*80*)	1923	17.29	
Nasmith (*89*)	1902	17.46		Cross and Swain (*78*)	1924	17.53	93.6°
Osborne and Harris (*92*)	1903	17.66		Tague (*98*)	1925	17.51	
Osborne and Harris (*93*)	1903	----	−92.28°	Dill and Alsberg (*79*)	1925	17.54	−91.0°
König and Rintelen (*84*)	1904	17.77		Blish and Sandstedt (*77*)	1926	17.54	
Mathewson (*88*)	1906	17.47	−91.8°– −93.1°	Jones and Wilson (*66*)	1928	17.81	

[1] With the exception indicated, the determinations were made on gliadin in 70 percent ethyl alcohol.
[2] This determination was made with gliadin in 45 percent alcohol.

TABLE 3.—*Nitrogen content of glutenin prepared from wheat of different varieties and origin as determined by various investigators*

Investigator	Year	Nitrogen	Investigator	Year	Nitrogen
		Percent			*Percent*
Ritthausen (*97*)	1872	17.14	Tague (*98*)	1925	17.39
Osborne and Voorhees (*95*)	1893	17.19	Cross and Swain (*78*)	1924	16.81
Osborne and Harris (*92*)	1903	17.49	Jones and Moeller (*61*)	1928	16.82

Cross and Swain (*78*) made a study of gliadin and glutenin prepared from flour obtained from different varieties of wheat and from wheat grown in different geographical locations. They conclude that "the evidence offered points to the chemical identity of the gliadins from the different varieties of wheat."

In a comparative study of the glutelins of cereal grains Larmour (*85, p. 1093–1094*), concludes—

from the papers cited in the foregoing discussion it seems evident that glutenins from various types of flours are indistinguishable by present methods of chemical analysis, and by means of the measurements of specific rotation, refractive index, and absorption spectra.

Blish (*75*) could not differentiate chemically between a glutenin prepared from a high-grade patent flour and that prepared from a biscuit flour.

Blish and Pinckney (*76*) found that samples of gliadin and glutenin from widely different types and varieties of wheat were identical, with the exception of slight differences noted in optical rotation in the case of the proteins from one unusual variety of wheat.

What has been said regarding the constancy of the composition of gliadin and glutenin, irrespective of the class or source of the wheat from which they were obtained, can also be applied to the proteins of other materials. Arachin, for example, a protein of the peanut, has definite physical and chemical properties, irrespective of the environmental conditions under which peanuts are grown. Edestin, from hempseed, is a crystalline protein having definite physical and chemical properties. Preparations of edestin obtained by different workers and from hempseed grown in different parts of the world throughout a period of many years have been found to have the same elementary composition.

The overwhelming evidence that the chemical composition of individual proteins is not influenced by environmental conditions under which the plants containing them were grown justifies the use of nitrogen conversion factors based on the nitrogen content of individual proteins.

On the basis of the properties and composition of their proteins, most of our common, nitrogenous foodstuffs can be classed in five general groups: Cereal grains, oilseeds and nuts, seeds of leguminous plants, foods of animal origin, and fruits and vegetables. For the sake of convenience in presentation, the nitrogen conversion factors for the proteins in a number of different food materials are considered under those classes.

It is realized that many food articles are not included among those for which special factors are given. Special factors are suggested only for those articles concerning the proteins of which it is believed there is sufficient information to justify the use of special factors.

PROTEINS OF CEREAL GRAINS

WHEAT

The factor generally used for wheat flour and for the whole wheat kernel, 5.7, is based on the nitrogen content of the two principal proteins in flour, namely, gliadin and glutenin.

However, flour has a protein content about 1 percent lower than that of the wheat from which it is obtained. This has been assumed to be due to the fact that bran contains a higher percentage of protein than the endosperm. Work done in this Bureau on the proteins of wheat bran has shown that this is only one cause. The other cause of the lower protein content of the flour as compared with that of the wheat from which it is made is that the proteins of bran have not the same nitrogen percentage as the proteins of flour. Two of them have a lower percentage. The nitrogen of bran represents approximately 22 percent of the total nitrogen of wheat. From a commercial wheat bran from which nearly all the adhering parts of the starchy endosperm had been removed by special treatment, three proteins were isolated, an albumin, a globulin, and a prolamin. These proteins were obtained in percentages of 2.87, 2.35, and 5.35, respectively, which amounted collectively to 10.57 percent of the bran. They have been studied extensively with regard both to their chemical composition and properties and to their nutritive value.

The results of these studies have raised the question whether or not a factor based not only on the nitrogen content of gliadin and glutenin, but also taking into account the proteins of the bran and the embryo, would give the protein content of wheat more accurately than the customary factor 5.7. Special interest lends itself to this matter in view of the current practice of buying wheat upon analysis and determination of its protein content. Accordingly, the factors have been calculated for converting the percentages of nitrogen in the endosperm, embryo, and bran into terms of protein. On the basis of the nitrogen percentages in these different parts of the seed and of their relative distribution in the wheat kernel, a factor has been obtained for the conversion of the nitrogen of the whole kernel into terms of protein.

ENDOSPERM

The proteins of the endosperm consist chiefly of gliadin and glutenin, which occur in wheat in approximately equal quantities. Although traces of a globulin and albumin have also been isolated from flour, it is questionable whether these small quantities found in the best flour may not be due to the presence of small quantities of embryo that escape separation in the milling process. At any rate, the quantities are too small to affect the results significantly when considering the conversion factors for the endosperm nitrogen. Gliadin contains about 17.6 percent of nitrogen, and glutenin about 17.5. The endosperm proteins collectively, therefore, contain 17.55 percent of nitrogen, which, divided into 100, gives the conversion factor 5.698, or 5.7, which is the factor generally used for the conversion of nitrogen into protein percentage not only in flour, but also in whole wheat.

BRAN

The proteins of bran have been shown (*46*) to consist essentially of an albumin, a globulin, and a prolamin. The proportions in which these proteins have been isolated from bran and the percentages of nitrogen which they contain are shown in table 4.

TABLE 4.—*Proteins of wheat bran and their nitrogen content*

Protein	Nitrogen	Quantity of protein isolated from 100 grams of bran
	Percent	*Grams*
Albumin	15.4	2.87
Globulin	17.7	2.35
Prolamin	15.3	5.35
Total		10.57

On the basis of these data, the bran proteins collectively contain 15.86 percent of nitrogen, corresponding to the conversion factor 6.31.

EMBRYO

Osborne and Campbell (*91*) obtained from wheat embryo about 10 percent of albumin, 5 percent of globulin, and 3 percent of proteose, which contain 16.8, 18.3, and 17.0 percent of nitrogen, respectively. Calculated on the basis of these figures, the embryo proteins as a whole contain 17.24 percent of nitrogen, corresponding to the conversion factor 5.80.

WHOLE WHEAT KERNEL

In their classic work on the nutritive value of the wheat kernel and its milling products, Osborne and Mendel (*94*) have estimated the percentages of the total protein of the wheat kernel which is contained in the endosperm, bran, and embryo as follows: Endosperm, 73.3 percent; bran, 22.3 percent; embryo, 4.4 percent.

These figures are based on approximate proportions of the above-named parts in the average moisture-free wheat kernel containing 2.2 percent of nitrogen. The authors state: "The relative proportion of these parts varies somewhat in different samples of wheat, but in general we believe that these figures fairly represent the average."

By taking into account the foregoing proportions of the parts in the seed, the relative quantities of the different proteins which have been isolated from these parts, and the percentages of nitrogen in the proteins, the conversion factor for the nitrogen of the whole wheat kernel may be calculated in the following manner:

Total nitrogen in 73.3 grams of endosperm proteins:

	Grams
73.3×0.1755	12.864

Total nitrogen in 22.3 grams of bran proteins:

$$\frac{2.87\times 22.3\times 0.154}{10.57}=0.9325 \text{ gram albumin nitrogen.}$$

$$\frac{2.35\times 22.3\times 0.177}{10.57}=0.8775 \text{ gram globulin nitrogen.}$$

$$\frac{5.35\times 22.3\times 0.153}{10.57}=1.7269 \text{ grams prolamin nitrogen.}$$

Total	3.537

Total nitrogen in 4.4 grams of embryo proteins:

$$\frac{10\times4.4\times0.168}{18}=0.411 \text{ gram albumin nitrogen.}$$

$$\frac{5\times4.4\times0.183}{18}=0.224 \text{ gram globulin nitrogen.}$$

$$\frac{3\times4.4\times0.17}{18}=0.125 \text{ gram proteose nitrogen.}$$

	Grams	
Total	0.760	
Total nitrogen in 100 grams of total wheat proteins		17.161

$100\div17.161=5.83$ conversion factor.

The conversion factor 5.83 thus obtained is based partly on the relative proportions in which the proteins have been isolated from the different parts of the seed. The quantities actually isolated, however, probably do not represent all the protein present, especially in the embryo and the bran. It is seldom that the proteins in a naturally occurring plant or animal product can be completely extracted. Furthermore, more or less unavoidable losses are always involved in the separation and isolation of the proteins after they have been extracted from the material which originally contained them. Such losses are usually, however, rather uniformly distributed among the different proteins of the seed and would therefore not significantly affect the conversion factor. Although the new factor here presented for the conversion of the percentage of nitrogen of wheat into terms of protein does not differ greatly from the factor in general use, it is believed that it will give the protein content of wheat more accurately than the customary factor 5.7.

RYE, BARLEY, AND OATS

The proteins of these cereals, like those of wheat, have been found to consist chiefly of an alcohol-soluble protein (prolamin) and a glutelin. Several investigators have shown that the nitrogen content of these proteins agrees quite closely with that of gliadin and glutenin, the corresponding proteins of wheat. Like wheat, these cereals contain other proteins, but their quantity is proportionately so small that they need not be considered in connection with the conversion factor. The factor 5.83, which has been calculated for wheat, can therefore also be used for rye, barley, and oats.

RICE

The proteins of rice differ essentially from those of other cereals, with respect both to the nature and properties of the proteins and to their amino acid composition. Rice contains but an insignificant quantity of protein belonging to the class of prolamins. Its protein content consists largely of oryzenin. Small quantities of two globulins are also present. If the relative proportions in which the different proteins are present in rice, and the percentages of nitrogen in these proteins are taken into consideration the nitrogen content of oryzenin, 16.8 percent, fairly represents the percentage of nitrogen in the rice proteins as a whole. This percentage corresponds to the conversion factor 5.95.

CORN (MAIZE)

The chief proteins of corn are zein and a glutelin. It also contains globulins in relatively small quantities. The percentages of nitrogen both in zein and in corn glutelin as recorded by numerous investigators are close to 16.1 percent, which practically gives the conversion factor ordinarily used, 6.25.

PROTEINS OF OILSEEDS AND NUTS

The predominating proteins in nearly all the oilseeds and nuts which have been studied are globulins of relatively high nitrogen content, ranging from 18.5 to 19 percent, corresponding in general to the factor 5.3. The proteins of the following food articles belonging to this class have been studied: Almonds, hazelnut, walnut, Brazil nut, butternut, coconut, castor bean, hempseed, cottonseed, sunflower seed, flaxseed, squash seed, pumpkin seed, sesame seed, and cantaloupe seed.

Although peanuts and soybeans are oilseeds, their predominating proteins have a somewhat lower nitrogen content than those of the oilseeds mentioned above. More than 87 percent of the total nitrogen of peanuts is protein nitrogen (*54*). The proteins consist almost entirely of two globulins, arachin and conarachin, both of which contain 18.3 percent nitrogen. This gives the conversion factor 5.46. In view of the definite knowledge of the proteins of the peanut, it is believed that the use of this factor will give quite accurately the real protein content of peanuts.

The chief protein in soybeans is glycinin, a globulin that has 17.5 percent nitrogen. This corresponds to the factor 5.71.

PROTEINS OF LEGUMINOUS SEEDS

Studies conducted in this Bureau and elsewhere, on the proteins of a number of different kinds of beans have shown that the percentages of nitrogen in the proteins of these beans lie close to 16 percent. The factor 6.25, therefore, can well be used for calculating the protein content of the following varieties of beans: Common white navy bean, Lima bean, adzuki bean, mung bean, velvetbean, and jack bean. In view of the insufficiency of data regarding the proteins of other leguminous seeds used for foods, it is recommended that the factor 6.25 be used tentatively for them also, with the exception of soybeans and peanuts, which are discussed under the heading oilseeds and nuts.

PROTEINS OF ANIMAL ORIGIN

MILK

From 75 to 80 percent of the nitrogenous matter of cow's milk consists of casein. Most of the remainder is in the form of lactalbumin. Milk also contains small quantities of a globulin and of a protein soluble in dilute alcohol. Casein and lactalbumin contain, respectively, about 15.9 and 15.4 percent nitrogen. The factor 6.38, which has been in general use for some time in estimating protein in milk, should be retained.

MEAT AND EGGS

The factor 6.25 is used in estimating the proteins of eggs and meat. Most of the proteins that have been isolated from animal tissue contain about 16 percent nitrogen.

PROTEINS OF FRUITS AND VEGETABLES

There is not sufficient knowledge regarding the proteins of fruits and vegetables to justify the calculation of a special factor. Until more is known regarding the proteins of this class of foods the conventional factor 6.25 should be used.

In table 5 are given the nitrogen-conversion factors for some food substances concerning the proteins of which, it is believed, there is sufficient knowledge to justify the use of special factors for converting the percentages of nitrogen into that of protein. Although it should be understood that these factors will not give absolutely correct values, for the reasons that have been already discussed, it is believed that they will give the true protein content more accurately than will the indiscriminate use of the conventional factor 6.25.

Table 5.—*Factors suggested for use in converting percentages of nitrogen in various substances into terms of protein* [1]

Substance	Factor suggested
Cereal grains:	
Wheat, endosperm	5.70
Wheat, embryo	5.80
Wheat, bran	6.31
Wheat, whole kernel	5.83
Rye	5.83
Barley	5.83
Oats	5.83
Rice	5.95
Corn (maize)	6.25
Oilseeds and nuts:	
Hempseed	5.30
Cottonseed	5.30
Sunflower seed	5.30
Flaxseed	5.30
Squash seed	5.30
Pumpkin seed	5.30
Sesame seed	5.30
Cantaloupe seed	5.30
Almonds	5.18
Coconut	5.30
Oilseeds and nuts—Continued.	
Brazil nut	5.46
Hazelnut	5.30
Walnut	5.30
Peanut	5.46
Soybean	5.71
Butternut	5.30
Castor bean	5.30
Substances of animal origin:	
Milk	6.38
Eggs	6.25
Meats	6.25
Gelatin	5.55
Leguminous seeds:	
Navy bean	6.25
Lima bean	6.25
Mung bean	6.25
Velvetbean	6.25
Adzuki bean	6.25
Jack bean	6.25

[1] The factor 6.25 is generally used in calculating the protein percentages of all the materials listed in this table with the exception of wheat, milk and gelatin, for which the following factors are sometimes used: Wheat, 5.7; milk, 6.38; gelatin, 5.55.

For comparison, in table 6 are shown the percentages of protein (N×6.25) in some common food materials as given in standard food-composition tables and the percentages obtained for the same material as calculated on the basis of the conversion factors given in table 5. There is a marked difference in the protein percentages in most cases. For gelatin, the difference is more than 10 percent. Nuts present differences of from 2 to 4 percent.

TABLE 6.—*Percentages of protein in some food materials calculated with the factor 6.25 as compared with the values derived when using corrected factors*

Food material	Protein based on factor 6.25 [1]	Protein based on special factors
	Percent	*Percent*
Almonds	21.0	17.4 (N×5.18)
Barley meal and flour	10.5	9.8 (N×5.83)
Bread from high-grade patent flour	8.7	7.9 (N×5.70)
Buttermilk	3.0	3.1 (N×6.38)
Butternuts, edible portion	27.9	23.6 (N×5.30)
Brazil nuts, edible portion	17.0	14.8 (N×5.46)
Cheese, American pale	28.8	29.4 (N×6.38)
Cheese, cottage	20.9	21.3 (N×6.38)
Coconut, edible portion	5.7	4.8 (N×5.30)
Coconut, prepared, as purchased	6.3	5.3 (N×5.30)
Filberts, edible portion	15.6	13.2 (N×5.30)
Flour, entire wheat	13.8	12.9 (N×5.83)
Flour, patent, bakers grade	13.3	12.1 (N×5.70)
Gelatin	91.4	81.2 (N×5.55)
Hickory nuts, edible portion	15.4	13.1 (N×5.30)
Milk, whole	3.3	3.4 (N×6.38)
Milk, skimmed	3.4	3.5 (N×6.38)
Milk, condensed	8.8	9.0 (N×6.38)
Oatmeal	16.1	15.0 (N×5.83)
Peanuts	25.8	22.5 (N×5.46)

[1] The percentages in this column are based on data for crude protein given by Atwater and Bryant (*74*).

The lower figures for gelatin and nuts are believed to represent closely the actual protein content. Gelatin is known to contain about 18 percent nitrogen, and most of the nitrogen in nuts occurs in the globulin fraction, which contains approximately 19 percent nitrogen. Although special conversion factors are sometimes used for a few materials, notably wheat and wheat products, milk and milk products, and gelatin, it should be emphasized that the factor 6.25 is the one that has been used in the preparation of nearly all food-composition tables that are in general use.

SUMMARY

The method used for estimating protein in foods and feeds by multiplying the percentage of nitrogen by the conversion factor 6.25 gives in many cases figures which are far from representing the correct values for real protein. This method rests on two assumptions, neither of which is necessarily correct; namely, that all the nitrogen in food material is protein nitrogen and that all proteins contain 16 percent nitrogen. Most naturally occurring nitrogenous substances contain nonprotein nitrogen. The nitrogen content of more than 121 different proteins isolated from plant and animal sources is given. In these proteins the nitrogen content ranges from 13 percent to more than 19 percent.

The magnitude of errors arising from the use of wrong conversion factors in estimating the protein content of foods is illustrated by citing the figures for a number of foods. The chief proteins in most of our common food materials have been isolated, and the percentage of nitrogen in these proteins is definitely known. On the basis of this knowledge, special nitrogen conversion factors have been calculated for certain foodstuffs concerning the proteins of which sufficient is known to justify the use of special factors. These foods

include some of the more important cereal grains, oilseeds and nuts, leguminous seeds, and substances of animal origin.

Although it is not claimed that these special factors will give absolutely correct values, it is believed that they will give the true protein content more accurately than will the indiscriminate use of the conventional factor 6.25.

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