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Professional paper

DETERMINATION OF PROTEINS BY THE KJELDAHL METHOD IN CEREALS OF THE MARKETS IN KORÇA CITY

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Abstract

Proteins are organic substances, macromolecules which mainly consist of the elements of carbon, hydrogen, oxygen, nitrogen, and rarely sulphide. Proteins are the main building blocks of cells. They not only give cells a structure, but are also molecular machines, where they transport materials, pump ions, catalyze chemical reactions and recognize signaling substances. The Kjeldahl Method is used to determine the protein content in cereals, a method invented since 1883, which consists of mineralizing the content of flour using Se and Cu_2SO_4 catalysts and burning the content with H_2SO_4cc for about 1 hour. After mineralization, it continues with distillation and finally with the titration of the distillate to determine the amount of acid needed to neutralize the distillate. This methodology is used to calculate the nitrogen content in cereals and then it is converted into protein content using conversion coefficients. In the study, samples of flour from different cereals of Korca city markets were taken for which the same analysis was performed and the final results are presented in the final table where buckwheat has the highest protein content of 17.59% and rye has the lowest content of 6.12%.

Keywords: Kjeldahl, protein, distillation, titration, catalyst, nitrogen.

1. Introduction

Proteins are organic substances, macromolecules which mainly consist of the elements of carbon, hydrogen, oxygen, nitrogen, and rarely sulfur. Proteins are the main building blocks of cells. They not only give cells a structure, but are also molecular machines, where they transport materials, pump ions, catalyze chemical reactions and recognize signaling substances.
Proteins are large molecules that consist of hundreds and thousands of elementary units - amino acids. Such substances, composed of repeating elementary units - monomers, are called polymers. Therefore, proteins can be called polymers, whose monomers are amino acids. There are many different types of proteins and each protein is specialized for a certain function. The structure of protein molecules, their synthesis and the realization of their biological function are the basic problems of biochemistry today. They have a very large molecular mass
Proteins, also called proteins (Greek protos - first), in animal and plant cells perform various and very important functions, which include the following.

Catalytic. Natural catalysts - enzymes are entirely or almost entirely proteins. Thanks to enzymes, chemical processes in living tissues are accelerated hundreds of thousands or millions of times.

Protective. Certain types of proteins protect the cell and the body as a whole from the entry of pathogens and foreign bodies. Such proteins are called antibodies. This mechanism of resistance to pathogens is called immunity.

Hormonal. Together with the nervous system, hormones control the work of various organs.

Reflective. Cell proteins carry out reception of signals coming from the outside. At the same time, various environmental factors (temperature, chemical, mechanical, etc.) cause changes in the structure of proteins - reversible denaturation, which, in turn, contributes to the

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occurrence of chemical reactions that ensure a response of cells to external irritation. This ability of proteins underlies the work of the nervous system, the brain.

The engine. All types of cell and body movements, cilia twitching in protozoa, muscle contraction in higher animals and other motor processes - are produced by a special type of protein.

Energy. Proteins can serve as a source of energy for the cell. In the absence of carbohydrates or fats, amino acid molecules are oxidized. The energy released in this process is used to support the vital processes of the body.

Transportation. The hemoglobin protein in the blood is able to bind oxygen from the air and transport it throughout the body. This important function is also characteristic of some other proteins.

Plastic. Proteins are the main building material of cells (their membranes) and organisms (blood vessels, nerves, digestive tract, etc.). At the same time, proteins have individual specificity, i.e., the organisms of particular people contain some proteins that are characteristic only for them.

Protein metabolism in the human body is very complex. Depending on the state of the body, the required amount of certain proteins changes constantly, proteins are broken down, synthesized, some amino acids pass into others or break down, releasing energy. As a result of the vital activity of the body, a part of protein is lost, this is usually about 25-30 grams of protein per day. Therefore, proteins must be constantly present in the human diet in the right amount.

The recommended daily protein intake is 0.75-0.80 grams of quality protein per kilogram of body weight for an adult, i.e. about 50-60 grams per day for an average male and 40-50 grams for a female. Children, especially very young ones, need more protein (up to 1.9 grams per kilogram of body weight per day) as their bodies grow rapidly. The protein content found in the literature for the cereals taken in the study is shown in Table 1.

Table 1: Summary table with the nutritional values of the cereals taken in the study

Nutrition values for 100 gr	Wheat	Corn	Rice	Oat	Buckwheat	Quinoa	Rye
Calory (Kcal)	170	361.8	218	364	343	366	338
Fat (g)	1.5	0.35	1.60	6.9	3.4	4	1.63
Carbohydrates (g)	32	80	45.85	66.3	71.5	54.4	75.9
Fibers (g)	4	5.3	3.5	10.6	10	14.6	6
Proteins (g)	9	6.44	4.55	16.9	13.3	8.9	8

2. Method and Materials

In 1883, Johan Kjeldahl introduced the "New Method for the Determination of Nitrogen in Organic Bodies", revolutionizing nitrogen analysis and setting new standards. He developed his method for protein analysis by using an empirical relationship between protein content and nitrogen content. Since then, the method has become indispensable in fields such as food analysis, soil analysis, and water analysis. However, it is by no means limited to these areas: This versatile method can also be found in the industrial or pharmaceutical sectors and wherever nitrogen content is important.

Thanks to the wide range of possible applications, high accuracy, and simple execution, the Kjeldahl analysis is still considered the reference method today. Because the Kjeldahl analysis can be used to determine all nitrogen compounds. In addition to total nitrogen content, individual compounds such as ammonium, nitrate, nitrite, and organically bound nitrogen can be determined from a wide variety of sample matrices.

The Kjeldahl analysis can be divided into 3 work steps that also form the basis of the classical application:

Digestion of samples with sulfuric acid: The method begins with heating a sample to 360-410 degrees Celsius with H_2SO4 (concentrated sulfuric acid). The acid dissolves in the organic sample by oxidation to release the reduced nitrogen as ammonium sulfate. As a result, hot concentrated sulfuric acid oxidizes carbon and sulfur. Catalysts such as selenium, Hg_2SO4 or $CuSO_4$, are added to speed up digestion. Also, Na_2SO_4 and K_2SO_4 are added to raise the boiling point of H_2SO4. When the drinks emit fumes, it is understood that the digestion is complete.

$$NCOC + H_2SO_4 \longrightarrow (NH_4)_2SO_4 + CO_2 + SO_2 + H_2O$$

.

As for the practical part, this analysis is automated as specific catalysts accelerate the decomposition. First, mercuric oxide was chosen as the catalyst. But even though it was effective, it was replaced by copper sulfate because it created a health problem. The latter was not as efficient as mercuric oxide and resulted in lower protein results.

Steam distillation: Immerse the end of the condenser in a known volume of acid with a known, standard concentration. In most cases, H_3BO_3 with an excess of ammonia is used. But HCl or H2SO4 or any other strong acid can also be used. Then the sample solution is distilled with a small amount of NaOH, which we add with a dropper. NaOH reacts with NH_4^+ to NH_3. From this process, ammonia reacts with the standard solution and other salts.

$$2NaOH + (NH_4)_2SO_4 \longrightarrow 2NH_3\uparrow + Na_2SO_4 + 2H_2O$$

$$2NH_3 + 4H_3BO_3 \longrightarrow (NH_4)_2B_4O_7 + 5H_2O$$

Distillate Titration: Titration is the method that measures the concentration of ammonium ions in the acid solution. With the use of boric acid, direct acid-base titration is done with a strong acid of known concentration. If HC1 or H_2SO_4 is used, indirect reverse titration is used: the strong concentrated base known as NaOH is used to neutralize the solution. In this case, the amount of ammonia is calculated as the difference between the amount of HCl and NaOH. When we have a direct titration, it is not necessary to know the exact amount of a weak acid, eg boric acid, because it does not interfere with the titration, it must be more than ammonia to capture it efficiently. So, in a standard solution, direct titration is needed, while for two, reverse titration.

$$(NH_4)_2B_4O_7 + 5H_2O + 2HCl \longrightarrow 2NH_4Cl + 4H_3BO_3$$

For a better understanding of how Kjeldahl analysis works with automated analysis systems, a diagram explaining the step-by-step process is provided below in figure 1.:

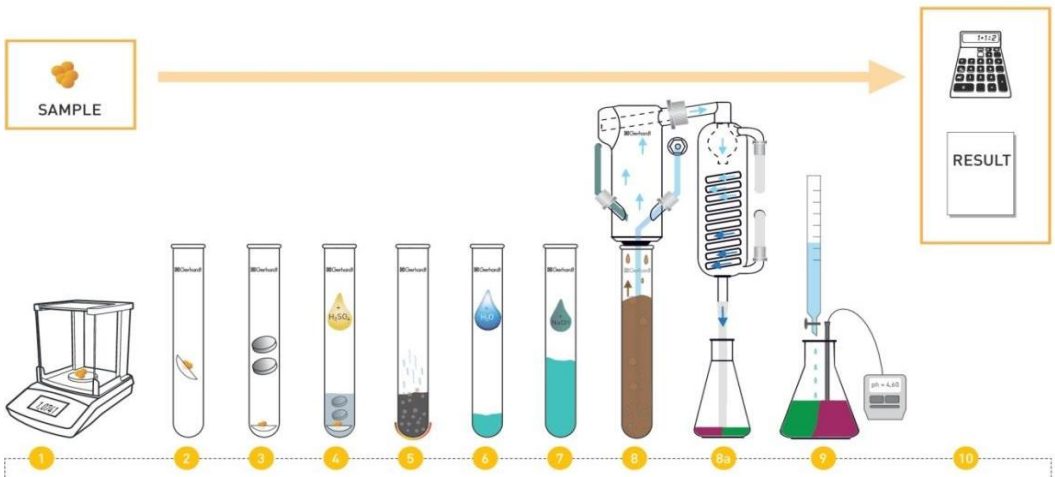


Figure 1: Diagram that explains step by step the automated processes of the Klejdahl Method

The sensitivity of the Kjeldahl method is noted in the original version. Many other methods have been used to detect the amount of NH_4^+ after mineralization and distillation, improving sensitivity. But this method also has limitations. This method is not applicable to compounds containing nitrogen in the nitro and azo groups and nitrogen present in the rings, since the nitrogen of these compounds does not dissolve in sulphat-ammonium under the conditions provided by this method.

First, the whole procedure starts with the collection of different types of flour from cereals to have some results so that we can make the final comparisons. The collection of raw materials was carried out in the markets of the Korça region. A total of about 24 samples of cereals of Albanian, Italian and German origin were collected, respectively, according to the markets found in the Korça region. About 2g of ground product, sieved in 2 mm holes and dried at 105 °C to constant weight, accurately weighed and quantitatively transferred to a digestion tube as shown in figure 2 a) and b).

Figure 2 a) and b): Weighing of samples (a) and determination of moisture on a thermal scale (b)

Reagents:

To each sample placed in the digestion tube were add:

1. 7 g anhydrous potassium sulfate (K_2S0_4)
2. 5 mg Selenium (Se) Powder
3. 12 ml sulfuric acid (H_2SO_4.) concentrated 96%
4. 5 ml Hydrogen Peroxide (H_20_2) 35%
5. HCl 0.2N
6. Indicator
7. H_3BO_3 4%

Mineralization: It is heated for 60 minutes at 420 °C as shown in figure 3. This action is carried out inside the cap as it is accompanied by a high release of sulfuric vapors.

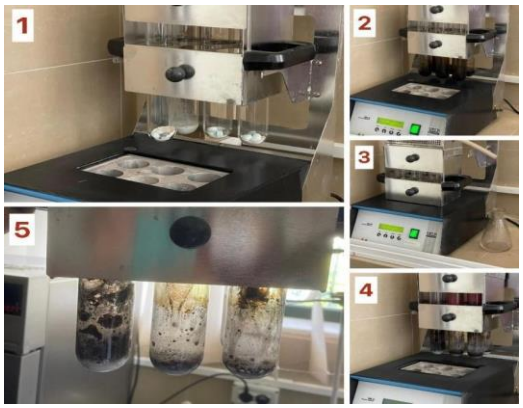


Figure 3: Protein mineralization process in a mineralizer with 6 parallel samples

Allow the digestion tubes to cool to 50-60 °C, and then add 50 ml of deionized water.

Distillation: Place the Erlenmeyer flask containing 25 ml of boric acid solution (4%) in position in the steam distillation unit. Place in position in the distillation unit a tube used for digestion with the dissolved sample and the dilution water as shown in figure 4 a) and b). Collect at least 100 ml of the sample in the Erlenmeyer flask. Addition of reagents: Na0H = 50 ml (35%).

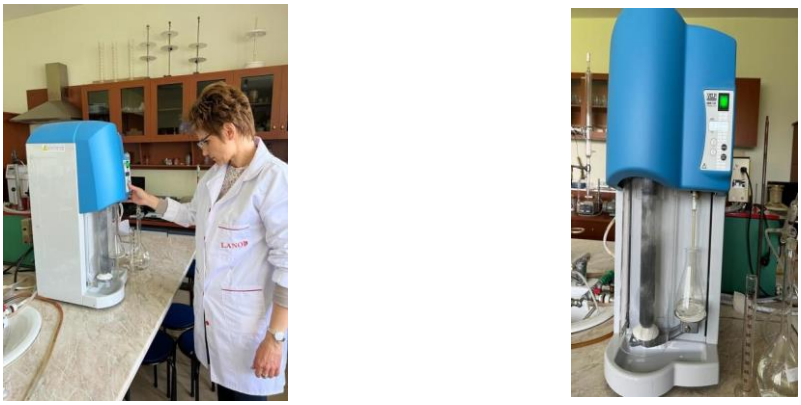


Figure 4 a) and b): Distillation process of samples (a) and Klejdahl still (b).

Titration: Add indicator solution (10 drops) which is prepared by mixing a content of 0.1 Blu-methylene and 0.04% Methyl red in ethanol and is otherwise known as Tashiros indicator and titrate with HCI 0.2 N as shown in figure 5.

Figure 5: Moment of titration of samples, transition from green color to purple color

Calculations: 25 mg of N-NH3 requires 8.92 ml of 0.2N acid (1ml of HC1 0.2 N = 2.803 mg of N-NH3)
For the preparation of HCl 0.2N, 16.36 ml of HCl is needed in 1000 ml of H20.
For calculation, the following formulas are used for the nitrogen content and for the amount of proteins using the coefficients for each cereal.

$$\%N = \frac{V \times 2.803}{W} / 10$$

$$\%P = \%N \times Cof$$

3. Results and Discussions

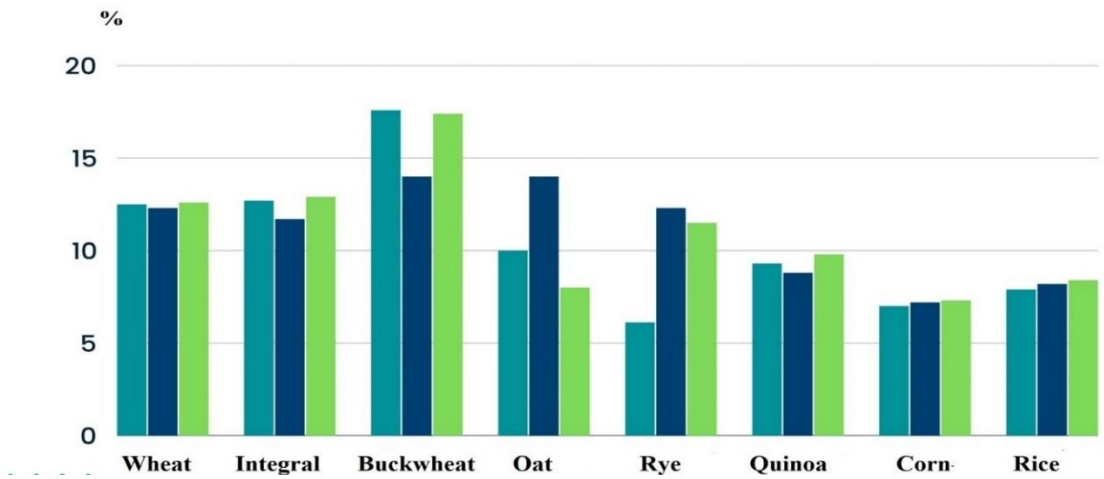
At the end of the experiment, we can affirm that the procedure was developed without problems, the mineralization and distillation were completely carried out giving their extracts and then the titration was accompanied by a color change from green to purple, an obvious color difference.

All the data gathered are shown below in the table 1. Index I stands for the samples gathered from cereals in Italian markets showing Italian origin and index G stands for German origin of cereals.

Table 1: Summary table with the results obtained from the experiment and from the above calculations

Sample	Type of Flour	W (g)	M (%)	Cof	V (ml)	N (%)	P (%)
1	Wheat	2	13.13	5.7	15.6	2.19	12.5
2	Integral	2	14.04	5.83	15.5	2.17	12.7
3	Buckwheat	2	13.31	6.31	19.9	2.78	17.59
4	Oat	2	10.87	5.86	15	2.10	12.32
5	Rye	2	10.31	5.83	7.5	1.05	6.12
6	Quinoa	2	8.35	5.75	11.5	1.62	9.3
7	Corn	2	12.25	6.25	8	1.12	7
8	Rice	2	8.82	5.95	9.5	1.33	7.9
9	Wheat I	2	12.8	5.7	15.4	2.15	12.3
10	Integral I	2	13.9	5.83	14.3	2.00	11.7
11	Buckwheat I	2	12.87	6.31	15.7	2.21	14.0
12	Oat I	2	10.67	5.86	14.7	2.06	12.1
13	Rye I	2	10.28	5.83	15.1	2.11	12.3
14	Quinoa I	2	8.67	5.75	10.9	1.53	8.8
15	Corn I	2	11.06	6.25	8.2	1.15	8.8
16	Rice I	2	8.63	5.95	9.8	1.38	8.2
17	Wheat G	2	13.2	5.7	15.7	2.21	12.6
18	Integral G	2	13.98	5.83	15.7	2.21	12.9
19	Buckwheat G	2	13.11	6.31	19.6	2.75	17.4
20	Oat G	2	10.48	5.86	15.2	2.13	12.5
21	Rye G	2	10.32	5.83	14.1	1.97	11.5
22	Quinoa G	2	8.67	5.75	12.1	1.70	9.8
23	Corn G	2	12.6	6.25	8.3	1.17	7.3
24	Rice G	2	8.9	5.95	10.1	1.4	8.4

Legend: W-weight M- Moisture Cof- Coefficient V- volume N- Percentage of N P- Percentage of Protein . I- Italian, G-German



Legend: Albanian Flour->…… Italian Flour->…….. German Flour->……

Graph 1: Comparison between the protein percentage of different origins and different types of flour from cereals in the markets of the Korce region.

4. Conclusions and Recommendations

We come to the conclusion that buckwheat is the cereal with the highest protein content around 17.59% and on the contrary rye is the one with the lowest content around 6.12%. These results go parallel to the theoretical results where the average protein content is 7-9%, wheat about 12% and the maximum protein content measured in cereals is 16%. But normally there are differences because always our experimental result depends on the type of sample taken in the study.

The particular advantage is the versatility of the matrices to be analyzed. For example, in addition to cereals, animal feed, dairy products or other foods, sludge, sewage sludge, composts and soils, as well as aqueous extracts and wastewater can also be analyzed for their nitrogen content. Especially with highly inhomogeneous sample materials, there is hardly any alternative to Kjeldahl due to high sample weights

One of the disadvantages of this method is its duration, mostly caused by the time needed for sample digestion (generally from 60 to 180 min). Various authors have proposed modifications to reduce this time: the use of electric heating to replace gas heating, modifications of the attack mixture that allows reducing the digestion time up to 10-15 minutes. Some analysts specifically use a mixture of sulfuric acid with phosphoric acid instead of sulfuric acid alone, others add oxygenated water. It is possible to increase the analysis possibilities by using a block of mineralization. This assembly consists of an aluminum block which is heated by an electrical resistance located at its base. This block is pierced by several holes shaped like test tubes to be used instead of KJELDAHL flasks. The substance that will be mineralized is placed with the attack liquid in the tube that goes above the hole in a refrigerator that serves to condense the vapors. The temperature of the block must be controlled by the thermostat. Some companies trade in a reduced space and with great speed and regularity. Other disadvantages are the necessary use of concentrated sulfuric acid at high temperature. These disadvantages have been compared with the Dumas method as unfavorable in the measurement of crude protein content.

Rice: You can consume rice 2-3 times a week, to make room for other grains in your daily diet. Prefer brown rice to white rice (except when rice is used as a treatment for intestinal discomfort, such as diarrhea, etc.). People suffering from diabetes should accompany it with fresh greens and not abuse its amount.

Rye: In addition to the aforementioned benefits, rye is also surrounded by disadvantages. If we have hypertension, the vast majority of products made with rye have no salt, and some, which are ultra-processed, have large amounts of salt. This is why its consumption is not recommended for people with hypertension.

Buckwheat: Recipes for it are different. You can boil it for about 10 minutes, strain it, and then use it in various cold salads, adding the vegetables you like, cheeses, or whatever you like! You can make it with soup, meatballs, or other forms similar to risotto recipes. Buckwheat can also be flour, so you can grind it and prepare dough for bread, crepes, various pies, etc.

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