Journal on Biology and Instruction **ISSN 2798-0863**
Vol. 3 No. 1 Tahun 2023 | 10 – 17

Determination of Protein Content of Processed Dairy Products Using the Kjeldahl Method

Silma Elvaretta Aska[1*], Alif Salma Nurillah[2], Kabira Benani[3], Nabila Wahyu Azizah[4]

[1,2,3,4] Industrial Chemical Engineering, Vocational, Sepuluh Nopember Institute of Technology
[1,*] Selvaretta5@gmail.com
*corresponding author

Article information

Article history
Received December 15, 2022
Revised June 17, 2023
Accepted June 30, 2023

Kata kunci:
Kjeldahl
Protein
Susu

ABSTRAK

Tiap proses pengolahan bahan makanan akan berpengaruh pada nilai gizi kandungan bahan tersebut, khususnya pada kandungan protein. Penelitian ini bertujuan untuk melihat pengaruh cara pengolahan produk olahan susu terhadap nilai proteinnya dengan menggunakan metode Kjeldahl. Metode yang digunakan dalam studi ini adalah kuantitatif dengan mempraktikkan metode Kjeldahl serta studi litaratur untuk memahami hasil di dalamnya serta melakukan komparasi terhadap hasil penelitian ini. Tahap analisis tahapan metode Kjeldahl ada 3 yaitu proses destruksi, destilasi, dan titrasi yang dilakukan oleh mesin Kjeldahl. Faktor konversi yang digunakan pun spesifik pada produk olahan susu yakni 5.85. Pengujian Kjeldahl ini menggunakan selenium sebagai katalis. Prinsip metode ini ialah mengoksidasi senyawa mengandung nitrogen yang dikonversi menjadi ammonia dan bereaksi dengan asam pekat yang membentuk garam amonium. Hasil menunjukkan bahwa kadar protein pada susu murni, susu bubuk, yoghurt, dan keju berturut-turut adalah 15.95%, 11.11%, 8.7%, dan 5.27%. Terbukti bahwa makin panjang proses pengolahan, terutama pada suhu yang tidak terkontrol, kadar protein menunjukkan nilai yang cenderung menurun.

Keywords:
Kjeldahl
Protein
Milk Product

ABSTRACT

Determination of Protein Content of Dairy Products with the Kjeldahl Method. Each food processing process will affect the nutritional value of the ingredients, especially the protein content. This study uses the Kjeldahl method to see the effect of processed dairy products dairy products on protein value. The method used in this study is quantitative by practicing the Kjeldahl method and studying literature to understand its results and make comparisons with the results of this study. There are three stages of the Kjeldahl method analysis: the process of destruction, distillation, and titration carried out by the Kjeldahl machine. The conversion factor used is also specific to dairy products, namely 5.85. This Kjeldahl test uses selenium as a catalyst. The principle of this method is to oxidize nitrogen-containing compounds, which are converted to ammonia and react with concentrated acids to form ammonium salts. The results showed that the protein content in whole milk, powdered milk, yoghurt, and cheese were 14.74%, 11.81%,

12.32%, and 5.27%, respectively. It is proven that the longer the processing time, especially at uncontrolled temperatures, the protein content tends to decrease.

INTRODUCTION

Milk is famous for its high protein content. Based on statistical data, around 500 million metric tons of milk are consumed in the world. Protein is the main component of biocatalyst enzymes that help metabolism in the body (Rosaini, Rasyid, & Hagramida, 2015). Protein in the body as an energy source is 4 kcal per gram, around 19% of the weight of meat and 45% of the body's muscle protein. Protein has the function of building and maintaining cells in body tissues. If the body experiences a protein deficiency, it can cause Kwashiorkor disease which usually occurs in babies or small children (Purnama, Winahyu, & Sari, 2019). Milk is known as a major source of high quality protein with various nutritional, functional and physiological activities. Milk is also a source of biologically active peptides (Wardani, Sujana and Nurul, 2020). Not only in the form of fresh milk, milk has many derivative products with different processing processes.

The processing of food ingredients will affect the nutritional value of the ingredients. One of the food processing processes is by using heating, fermentation, and so on. The heating process is a process with a temperature of 100°C or more with the aim of obtaining a delicious taste and killing microbes and inactivating all enzymes. Cooking using heating can be done by boiling and steaming, broiling (roasting meat), baking (toasting bread), roasting, and frying (frying in oil). All cooking methods can cause loss of nutritional content in food (Sundari, Almasyhuri, & Lamid, 2015).

Even though it can remove nutritional content, the cooking process can increase digestibility and reduce various anti-nutritional compounds. For example in protein nutrition, boiling raw soybeans and the soybean fermentation process in making tempeh. However, on the other hand, some food processes can increase nutrition, for example fermentation (Weng & Chen, 2011). Therefore, it is necessary to test the value of post-processing nutritional content analysis, one of which needs to be analyzed is the protein content.

One method of protein analysis is the Kjeldahl method. The Kjeldahl method has advantages compared to other methods, namely that it can be used to test nitrogen content roughly and the equipment used is simple. The Kjeldahl method can analyze crude protein levels in food ingredients because it uses nitrogen content calculations (Saragi, Andrie, & Taurina, 2021). Research to determine protein conversion specific to the type of food has been carried out. For example, dairy products have a conversion factor of 5.85 (Mariotti, Tome, & Mirand, 2008).

The general principle of the Kjeldahl method is to measure compounds containing nitrogen by oxidizing and converting them to ammonia. The ammonia then reacts with the concentrated acid to form ammonium salts. After that, base will be added to neutralize the reaction atmosphere and then distilled with a weak acid as a reservoir for the distillate. Analysis is carried out by titration for the amount of N to be converted using a conversion factor. There are 3 stages of the Kjeldahl method analysis, namely the destruction, distillation and titration processes carried out by the Kjeldahl machine (Sylvia, Apriliana, & Rasydy, 2021). The Kjeldahl method is a standard method used to determine protein with its universal properties of high precision and good reproducibility (Amalia & Fajri, 2020). Therefore, protein content analysis was carried out to analyze the influence of fermentation and heating processing processes on the protein content of dairy products include cheese, yoghurt, fresh milk and powdered milk.

METHOD

Tools

The tools used in this research were Bunsen, tripod, stand and clamp, Kjeldahl digestion tool, 100 ml measuring flask, funnel, spray bottle, 50 ml measuring cup, 1 set of distillation tools (round flask, condenser, connector, thermometer), 100 ml beaker, and burette.

Materials

The materials used in this research were concentrated H_2SO_4, selenium, sodium carbonate, boric acid, methyl red, spirit and distilled water. Apart from that, there were samples of milk, yoghurt, cheese and powdered milk.

Procedures

a. Material Preparation Stage

This research method consists of four stages, namely material preparation. 40% NaOH was made in 100 ml, 2% boric acid solution was made in 100 ml, 0.01 N sodium carbonate solution was made, H_2SO_4 became 0.01 N from 1 N stock solution, and standardization of H_2SO_4 with sodium carbonate was carried out.

b. Destruction Stage

15 ml of concentrated H_2SO_4 was taken, 0.5 gram of sample and 1 gram of selenium were weighed, the three ingredients were mixed in a Kjeldahl digestion apparatus and stirred gently, the Kjeldahl digestion apparatus was clamped on a stand, the Bunsen was placed underneath. The blower is turned on in the acid chamber and the Bunsen is lit with a match. The sample is waited until it changes color to clear green, the sample resulting from the digestion is poured into a 100 ml volumetric flask and diluted to the limit of the volumetric flask.

c. Distillation Stage

The distillation apparatus was prepared and 25ml of the sample that had been diluted was poured into it. Then 25 ml of the diluted sample and 25 ml of 40% NaOH solution were poured into a round flask, 15 ml of boric acid was poured into a 100 ml beaker and 5 drops of methyl red were added. 15 ml of boric acid is placed at the end of the condenser, ensuring that the end is immersed in boric acid. Then, the distillation process is carried out and the temperature does not exceed 90°C. Wait for the liquid in the container glass (beaker) until it reaches 35 ml.

d. Analysis Stage

10 ml of the distillate was taken, titrated with 0.01N H_2SO_4 which had been standardized with sodium carbonate. Titration and standardization were carried out in triplicate.

$$\%protein = \frac{(V1-V2)x\ Nx\ fk\ x\ dp}{W} \ldots\ldots (1)$$

Informaation:
W= Sample weight (mg)
V1= Volume of H2SO4 0.01 N sample titration
V2= Volume of H2SO4 blank titration
fk= nitrogen to protein conversion factor
fp= dilution factor

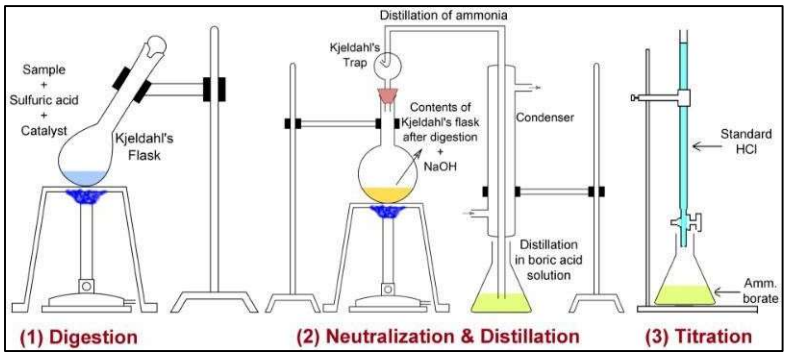

Figure 1. Scheme of the Kjeldahl Method

RESULTS AND DISCUSSION

Protein is the best source of nutrition for the growth of microorganisms, then these microorganisms will break down protein into foul-smelling metabolites, such as indole, cadaverine, organic acids, CO_2, H_2S, and skatole (Purnama, Retnaningsih, & Aprianti, 2019). Determination of total protein content quantitatively using the Kjeldahl method, where the determination of protein content is based on the nitrogen content contained in the material. Analysis of protein levels using the Kjeldahl method can basically be divided into three stages, namely the destruction stage, distillation stage, and titration stage (Purnama, Winahyu, & Sari, 2019).

The first stage is the destruction stage which aims to break down the contents of the sample into its elements. In order for the results obtained during digestion to be efficient, the heating during digestion must be high, exceeding 300°C, so that nitrogen and other elements can be released from their compound bonds (Purnama, Winahyu, & Sari, 2019). Therefore, heating with direct fire is very suitable, because the flame temperature resulting from Bunsen burning can reach 500°C. The sample is heated in concentrated sulfuric acid so that the sample decomposes into its elements, namely the elements C, H, O, N, S and P.

To speed up the destruction process, a catalyst is often added. By adding this catalyst, the boiling point of sulfuric acid will be increased so that destruction is faster. The catalyst provided is the addition of selenium. Selenium can speed up the oxidation process because apart from raising the boiling point, this substance also easily changes from low valence or vice versa. The function of sulfuric acid is to fix nitrogen and break down its elements. The N element in protein is used to determine the protein content in a material (Purnama, Retnaningsih, & Aprianti, 2019). The destruction process will produce carbon dioxide (CO_2), water (H_2O) and ammonium sulfate $(NH_4)_2SO_4$. The reaction that occurs is as follows:

$$(C,H,O,N) + O_2 + H_2SO_4 \rightarrow CO_2 + H_2O + (NH_4)_2SO_4$$

After the sample is heated at the digestion stage, when it has boiled and the sample changes color to a clear or transparent color, the distillation process will then be carried out (Erwansyah, Andrie, & Taurina, 2021). At this distillation stage, NaOH solution is added. The function of adding NaOH is to provide an alkaline atmosphere because the reaction cannot take place in acidic conditions. At this distillation stage, ammonium sulfate is broken down into ammonia (NH_3) by adding NaOH with alkali and heated in a distillation apparatus. The sample solution that has been destroyed is inserted into the distillation apparatus and placed on the left. Then a distillation device in the form of a long, small pipe is inserted into it until it almost reaches the bottom of the tube. So, it is hoped that the distillation process will run optimally (perfectly).

Boric acid (H_3BO_3) functions as a catcher for NH_3 as a distillate in the form of an alkaline gas. So that ammonia can be captured optimally, it is best to dip the tip of the distillation tool completely into a standard acid solution so that the amount of protein can be determined according to the protein content of the material (Nisah, Afkar, & Sa'diah, 2021). To support this, the distillation process during testing was carried out in a vacuum with the distillation tip directly connected to a closed distillate collection flask.

As the distillation process takes longer, the boric acid solution will change color from pink to green because the solution captures the presence of ammonia in the alkaline material, thus changing the pink color to green. The distillation reaction will end when the ammonia that has been distilled no longer reacts.

The reactions that occur in the distillation stage are as follows:

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

NH_3 is produced in the distillate in the form of gas. The NH_3 gas is captured by boric acid. The reaction is as follows:

$$4NH_3 + 2H_3BO_3 \rightarrow 2(NH_4)2BO_3 + 2H_2$$

The final stage is the titration stage. Titration at this stage is carried out to find out how much volume of H_2SO_4 is needed to change the color of the environment from green to pink. Before titrating the sample, 0.01N sulfuric acid was standardized with 0.01N sodium carbonate. So, the sulfuric acid concentration is 0.011. The titration process is the final stage of the entire Kjeldahl method for determining protein levels. This process aims to determine the nitrogen content in the sample through titration where the collected distillation results are titrated using H_2SO_4 (Nisah, Afkar, & Sa'diah, 2021).

Table 1. Sample test results using the Kjeldahl method

No	Sample	Titration Volume	N Total	Protein Percentage	SNI
1.	Whole milk	15	0.027267	15.95101864	Min. 2,8%
2.	Yoghurt	11	0.019004	11.11737663	Min 2.3%
3.	Powdered milk	9	0.023135	8.70055562	Min 2.7%
4.	Cheese	6.6	0.009014	5.273064014	-

Based on the three stages above using the Kjeldahl method, the protein content results in samples of whole milk, powdered milk, yoghurt and cheese in Table 1 are 15.95%, 11.11%, 8.7% and 5.27% respectively. At a glance it appears that the complexity of the process influences the test results.

According to Nurul (2020), the quality of milking protein is relatively constant. This is caused by several internal and external factors, each of which contributes quite significantly. Internal factors include physiological conditions, breed, lactation level, estrus, pregnancy, calving interval and age. The process of milk protein synthesis occurs in alveolar epithelial cells and is controlled by genes containing DNA. The process is by combining several amino acids to form protein. Some protein synthesis occurs in ribosomes which are bound to the double membrane of the endoplasmic reticulum, but some is located freely in the cytoplasm (Nugraha, Salman, & Hernawan, 2016). In order to be consumed, milk from cows needs to be pasteurized, which is a process of sterilizing food ingredients from microorganism contaminants. This can be concluded that the total protein content obtained from fresh milk (whole milk) has not been heated several times so that the protein is still high and does not experience significant damage even though some of the protein has been broken down into acid compared to the types of skim milk and full cream milk, but all treatments still meets the requirements of SNI for yoghurt of at least 3.5% (Syainah, Novita, & Yanti, 2014).

Yoghurt is a fermented milk product produced through the activity of lactic acid bacteria with the final microorganisms having to be active and abundant (Chairunnisa, 2019). The protein content in powdered milk can change due to processing factors when it is produced in a factory or during processing when it is consumed by consumers (for example: contaminated water when mixing milk, contaminated milk containers, etc.) (Sinaga, Maimunah, & Sitompul, 2021). Based on the analysis results of Weng & Chen (2011), the fermentation process can increase the protein content. The longer the fermentation time, the higher the concentration. This correlates with increased bacterial growth.

Cheese made with 0 day preservation is called cheese, the protein content of cheese ranges from 12.7-21% and cheese made with preservation has a protein content of 20.8-26.11%. The protein content of cheese increased with increasing concentrations of *M. miehei* used. Based on the results of cheese dry matter testing, there was a tendency for cheese dry matter to increase along with increasing concentrations of *M. meiehi*. This can be seen from the cheese yield which tends to increase with the addition of *M. meiehi* to milk when the milk pH reaches 6, because the optimum pH of *M. miehei* to produce protease enzymes is 5.5-7.5 (Mulyani, Azizah, & Legowo, 2009).

The decrease in protein levels obtained can be caused by several factors, such as heat, growth of microorganisms, acids, bases, organic solvents, pH, salt, heavy metal, and radioactive radiation. In milk samples, the decrease in protein levels can be caused by the milk being stored outside the refrigerator so that if it is left in the open, the protein quality can decrease. Apart from that, it can also be caused by microorganisms. Microorganisms can come from unclean tools and unclean surroundings (Purnama, Retnaningsih, & Aprianti, 2019). Meanwhile, the decrease in protein content in cheese is caused by the influence of protein hydrolysis by the renin enzyme into proteose, peptone, and amino acids during ripening. Apart from that, the curing process also affects protein levels. If the curing process is short, the protein levels obtained will decrease (Chairunnisa, 2007).

The reduction in protein levels in yoghurt depends on the lactic acid bacteria contained. If lactic acid levels are low, protein levels will decrease. Apart from that, the fermentation process can also reduce protein levels because there is Lactic Acid Bacteria (LAB) catabolism which breaks down protein into polypeptides, so that the protein is hydrolyzed into soluble components for the purpose of forming LAB cell protein (Nuraeni, Purwasih, & Romalasari, 2020).

CONCLUSION

From the results of the research above, it can be concluded that the protein content in whole milk, powdered milk, yoghurt and cheese is 15.95%, 11.11%, 8.7% and 5.27% respectively. It is proven that the longer and more complex the processing process, especially at uncontrolled temperatures, the protein content tends to decrease in value. However, all samples still meet SNI standards.

ACKNOWLEDGEMENT

Thanks are given to Saidah Altway, S.T., M.T., M.Sc. who has pioneered this research. Thanks to the Department of Industrial Chemical Engineering, Sepuluh Nopember Institute of Technology for providing support in the form of equipment and material loan facilities.

REFERENCES

Amalia, D., & Fajri, R. (2020). Analisis kadar nitrogen dalam pupuk urea prill dan granule menggunakan metode Kjeldahl. *Jurnal Kimia Sains dan Terapan*, 28-32.

Chairunnisa, H. (2007). Aspek nutrisi dan karakteristik organoleptik keju semi keras gouda pada berbagai lama pemeraman. *Jurnal Ilmu Ternak UNPAD, 7*(1), 16-21.

Chairunnisa, H. (2019). Addition of full cream milk powder in the production of fermented drink made from sweet corn extract. *Jurnal Teknologi dan Industri Pangan, 20*(2), 96-101.

Erwansyah, E., Andrie, M., & Taurina, W. (2021). Penetapan kadar protein sediaan salep fase air ekstrak ikan gabus (Channa striata) dan minyak cengkeh (Syzygium aromaticum L.) dengan metode biuret. *Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN*.

Mariotti, F., Tome, D., & Mirand, P. P. (2008). Converting nitrogen into protein—Beyond 6.25 and Jones' factors. *Critical Reviews in Food Science and Nutrition*, 177-184.

Mulyani, S., Azizah, A., & Legowo, A. M. (2009). Profil kolesterol, kadar protein, dan tekstur keju menggunakan *Mucor miehei* sebagai sumber koagulan. In *Prosiding Seminar Nasional Kebangkitan Peternakan-Semarang*, Fakultas Undip Semarang, 516-523.

Nisah, K., Afkar, M., & Sa'diah, H. (2021). Analisis kadar protein pada tepung jagung, tepung ubi kayu dan tepung labu kuning dengan metode Kjeldahl. *Amina, 1*(3), 108-113. https://doi.org/10.22373/amina.v1i3.46

Nugraha, B. K., Salman, L. B., & Hernawan, E. (2016). Kajian kadar lemak, protein, dan bahan kering tanpa lemak susu sapi perah Freis Holland pada pemerahan pagi dan sore di KPSBU Lembang. *Jurnal Universitas Padjajaran*.

Nuraeni, S., Purwasih, R., & Romalasari, A. (2020). Analisis proksimat yoghurt susu kambing dengan penambahan jeruk bali (Citrus grandis L. Osbeck). *Jurnal Ilmiah Ilmu dan Teknologi Rekayasa, 2*(1). https://doi.org/10.31962/jiitr.v2i1.36

Planten, J. T., & Hoppenbrouwers, R. (1954). Experiments on intra-ocular hyaluronidase administration. *Ophthalmologica, 127*(2), 17-121. https://doi.org/10.1159/000301932

Purnama, R. C., Retnaningsih, A., & Aprianti, I. (2019). Comparison of the protein content of UHT full cream liquid milk at room temperature storage and refrigerator temperature with variations in storage time by the Kjeldahl method. *Jurnal Analis Farmasi, 4*(1), 50-58.

Purnama, R. C., Winahyu, D. A., & Sari, D. S. (2019). Analisis kadar protein pada tepung kulit pisang kepok (Musa acuminata balbisiana Colla) dengan metode Kjeldahl. *Jurnal Analis Farmasi, 4*(2), 77-83.

Rosaini, H., Rasyid, R., & Hagramida, V. (2015). Penetapan kadar protein secara Kjeldahl beberapa makanan olahan kerang remis (*Corbiculla moltkiana* Prime) dari Danau Singkarak. *Jurnal Farmasi Higea*.

Saragi, R. R., Andrie, M., & Taurina, W. (2021). Penetapan kadar protein sediaan salep fase air ekstrak ikan gabus (*Channa striata*) dan madu kelulut (*Trigona* sp.) dengan metode Kjeldahl. *Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN, 5*(1), 2-8. https://jurnal.untan.ac.id/index.php/jmfarmasi/article/view/50170/75676590931

Sinaga, E. M., Maimunah, S., & Sitompul, E. (2021). Penetapan kadar protein dalam susu formula bayi. *Jurnal Ilmu dan Teknologi, 6*(1), 30-34.

Sundari, D., Almasyhuri, & Lamid, A. (2015). Analysis of protein levels in kepok banana skin flour. *Komposisi Zat Gizi Bahan Pangan Sumber Protein*, Media Litbangkes, 235-242.

Syainah, E., Novita, S., & Yanti, R. (2014). Kajian pembuatan yoghurt dari berbagai jenis susu dan inkubasi yang berbeda terhadap mutu dan daya terima. *Jurnal Skala Kesehatan, 5*(1), 48-58.

Sylvia, D., Apriliana, V., & Rasydy, L. O. (2021). Analisis kandungan protein yang terdapat dalam daun jambu biji (Psidium guajava L.) menggunakan metode Kjeldahl & spektrofotometri UV-Vis. *Jurnal Farmagazine*, 64-72.

Wardani, D., Sujana, D., & Nurul. (2020). Pemeriksaan kadar protein pada susu sapi segar asal peternakan Cilawu Kabupaten Garut dengan metode Kjeldahl. *Jurnal Sains dan Teknologi Laboratorium Medik, 5*(2), 18-22.

Weng, T. M., & Chen, M. T. (2011). Effect of two-step fermentation by *Rhizopus oligosporus* and *Bacillus subtilis* on protein of fermented soybean. *Food Science and Technology Research*, 393-400.