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# Protein Determination in some Animal Products from Sulaymaniyah Markets Using Kjeldahl Procedure

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## ABSTRACT

In this study, the crude protein of animal product samples was measured through determination of total nitrogen content using the Kjeldahl method. Measurement of total nitrogen by Kjeldahl procedure is the universal reference method for estimation of protein content in animal and plant products and is used for both calibration and validation of alternative methods in protein determination. The animal protein sources analysed in this study were samples of veal meat, chicken meat, egg and beef hot dog. Based on the results found in this research, the sequence of protein content in the samples was: chicken breast> fresh silverside > fresh thigh > frozen silverside > egg-yolk > beef hot dog > egg-albumin. We also concluded that the health claim of protein percentage that declared on the can of beef hot dog, as well the results of egg-albumin and fresh thigh chicken showed no significance differ compared to the standards.

***Keywords:*** Protein, Kjeldahl method, red meat, chicken, egg, beef hot dog.

## INTRODUCTION

Proteins are the most abundant constituent in biomolecules, making up 50% or more of their dry weight. Every protein has a unique structure and conformation or shape, which enables it to carry out a specific function in a living cell (Vaclavik *et al*., 2008) and differ from each other according to the kind, number and sequence of the amino acids (Mihaljev *et al*., 2015).

Protein is one of the most important essential nutrients in animal tissues to maintaining a healthy life, whereas the insufficient supply of proteins can result in health disorders such as marasmus, kwashiorkor, organ failure, and a weak immune system (Omotayo *et al*., 2016).

Proteins contain Carbon, Hydrogen, Oxygen, and Nitrogen as the main element of the amino acids while Sulfur and Phosphorus are minor constituents, however, Nitrogen is primary characteristic of proteins. All proteins are polymers of amino acids (Vasudevan *et al*., 2013), containing nitrogen on average 16% by weight. Out of 20 amino acids that present in proteins of animal origin, the human body can synthesize only 10 of them which are (Tyrosine, glutamine, serine, alanine, glycine, cysteine, aspartic acid, glutamic acid, proline and asparagine). The remaining 10 amino acids including essential amino-acids, encompass valine, isoleucine, lysine, leucine, arginine, histidine, methionine, tryptophan, threonine and phenylalanine, are essential to human health, as the body cannot synthesize (McClements, 2003), so the intake has to be through food to meet the needs of the organism (Dow *et al*., 1996).

The protein content in foodstuff is an important and essential parameter to determine the quality and safety of food (Birghila *et al*., 2015). Therefore, a credible analytical method, as well as choosing a suitable technique from the available methods is essential. In protein analysis, many criteria are considered, involving the nature of the protein, the presence of interfering substances, and the preferred speed, accuracy, and sensitivity of the assay (Martina and Vojtech, 2015, Wilson and Walker, 2010).

Methods currently used for the determination of the protein content in foods depend on the determination of nitrogen, and an empirically determined factor is subsequently used to convert the nitrogen content into the protein content(Mariotti *et al*., 2008).

The main objective of this study was to estimating protein in animal products and comparing these protein values with the standards or the values declared on the product's label to verify the validity of its claim.

## MATERIALS AND METHODS

### Sample preparation

Frozen and fresh red meat (veal), hotdog, fresh chicken meat was purchased in local supermarkets in Sulaymaniyah, Iraq. Samples were taken in triplicates from each part of the animal's body and the fresh samples were kept at (4°C) while the frozen was kept at (-18°C) until used. Egg sample (Turkish egg/ white color) with three replicates and kept at (4°C) until analyses.

### Moisture determination in the samples

Moisture content was determined according to method 985.29 of the AOAC International (William, 2000);. The exact weight of the samples was taken from 50g of (red meat, chicken, beef hot dog), after cutting meats into small slices, they were placed in petri dishs of known weight. Meanwhile, the eggs were broken, the yolks and

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albumins were separated and placed in separate petri dishes of known weight.

The percentage of moisture content was calculated according to the following formula:

**Moisture content % = [(g moist sample - g dry sample) / g moist sample]×100**

Also, the total solids of samples were calculated according to the following equation:

**Total solids % = 100 – Moisture content %**

*After the determination of moisture, the protein content was determined in all the samples using Kjeldahl procedure.

**Determination of protein by Kjeldahl method**

**Reagents preparation:**

1. **Sodium hydroxide solution, 40%:** 200g of sodium hydroxide was weighed and dissolved in distilled water. The volume was made up to 500mL in a volumetric flask.
2. **Boric acid solution 4%:** Weighing 20g of Boric acid and dissolved in distilled water, making up the volume to 500mL in a volumetric flask.
3. **Methyl red / bromocresol green indicator solution:** Dissolving 100mg methyl red and diluting to 100mL in 95% ethanol. Followed by dissolving 100mg Bromocresol green and diluted to 100mL in 95% ethanol. Finally mixing one part of the methyl red solution with five parts of the Bromocresol green solution.
4. **Blank:** Contains all the reagents used except for the sample in every batch, so to subtract reagents effects from the sample nitrogen (Puwastien *et al*., 2011).

**Procedure**

Protein total nitrogen content was determined in 1 gm of the dried samples using micro- Kjeldahl technique. The Kjeldahl method was performed according to method 981.10 of the AOAC International (Latimer, 2016); (William, 2000, Persson, 2008, Puwastien *et al*., 2011). The three steps of the Kjeldahl method were carefully carried out in sequence as follows:

1) **Digestion:** weighing about 1g in triplicate of the dried samples (red meat, chicken, Beef hot dog and egg) , into the Kjeldahl flask, followed by the addition of 30mL of concentrated sulphuric acid (95-97%), 0.4g of copper sulfate, and 3.5g of potassium sulfate. The mixture was heated in a fume cupboard slowly to prevent excessive frothing; then, the digestion was continued at 400°C for 2.5-3 hrs until the color of the mixture changed to iridescent blue color. The solution was left to cool down and diluted with distilled water to 100mL.
2) **Distillation:**10 ml of the digested solution was carefully added to 10 ml NaOH (40%) and fixed to the distillation device.
3) In the ammonia receiving flask, 10mL of boric acid (4%) was added with three drops mixture of methyl red and Bromocresol Green dye. Collecting up to 25-30 mL in a receiving conical flask after the end of the distillation process.
4) **Titration:** the collected solution in the receiving conical flask was titrated with 0.1M of HCL, and the titre was recorded from which the amount of nitrogen content was measured according to this equation:

**% Nitrogen= [{mL (titre–B)×M HCl×dilution factor×14.007}/(mg sample×10)]×100 Eq.1**

**Where:**

**M HCL= Molarity of hydrochloric acid, B= Blank**

To convert dry weight to wet weight, the moisture content of the raw material (i.e., wet weight ) must be found. The conversion formula is:

**Dry weight (D.Wt) = wet weight (W.Wt)×[(100-moisture percentage)/100] Eq.2**

To convert from dry weight to wet weight:

**Wet weight (W.Wt)= dry weight (D.Wt)×[100/(100-moisure percentage)] Eq.3**

This formula is used to find protein % in the samples:

**% P = % N × CF Eq.4**

**Where:**

**% P = Protein , % N = Nitrogen , CF = Conversion Factor**

**Statistical analysis**

Data collected for all parameters were analyzed by a completely randomized design (CRD). The difference among mean was tested by Duncan's multiple range tests, according to $P \leq 0.01$ significance, and the result of statistical analysis results are shown as mean value and standard error in tables. The statistical calculations of the results were completed using the XLSTAT (2016) program.

## RESULTS AND DISCUSSION

**Results of determination of protein, moisture in animal products**

The results obtained in this study are the determination of moisture and protein in some animal products using Kjeldahl procedure (Table 1).

To each product sample, the mean value of analysis and standard error of the mean were measured. Sample constituent values were ranged from 11.59 ( Egg-Albumin ) to 22.01% (Fresh Chicken breast) for protein, containing 50.0 ( Egg-yolk ) to 87.6 ( Egg-albumin ) of moisture. From the results showen, it can be concluded that the standard error of the mean value is the highest (0.47) in frozen silverside (veal meat), while in beef hot dog recorded the lowest value (0.07).

**Table 1. Percentages of moisture and protein contents in some animal products.**

| Sample | Type | %Protein | | | %Moisture | |
|---|---|---|---|---|---|---|
| | | %Mean | Standards or declared value | SEM | %Mean | SEM |
| Chicken meat | Fresh, Breast | 22.01 | 24.00 | 0.17 | 76.5 | 0.17 |
| | Fresh, Thigh | 19.74 | 20.90 | 0.08 | 78.57 | 0.33 |
| Veal meat | Fresh, Silverside | 20.55 | 22.70 | 0.33 | 76.8 | 0.09 |
| | Frozen, Silverside | 19.08 | | 0.47 | 76.5 | 0.10 |
| Egg | Egg-Albumin | 11.59 | 12.00 | 0.10 | 87.6 | 0.25 |
| | Egg-Yolk | 14.73 | 16.10 | 0.29 | 50.0 | 0.55 |
| Beef meat | Hot dog | 12.88 | 13.00 | 0.07 | 73.9 | 0.17 |

***CV-Coefficient of variation = (standard deviation ÷ mean)*100**

***VI- variation interval (span) = max. value – min. value**

***SEM-Standard error of the mean**

Standard of Egg-Yolk, fresh breast and thigh chicken and veal meat according to (Agency, 2002), Standard of Egg-Albumin according to (Coultate, 2002).

**Results of protein content in chicken meat**

In fig. 1. the result shows that chicken breast contains highest (22.01 %) protein content, followed by chicken thighof protein content 19.74 %.Hence the protein content in selected chicken samples, have different protein contents. Our results of protein content in chicken breast and thigh showed higher than the results recorded by Omotayo *et al*. (2016), which could be due to using of different type of chicken used which is Hyline brown (Chen *et al*., 2016); or even due to using another part of chicken meat.

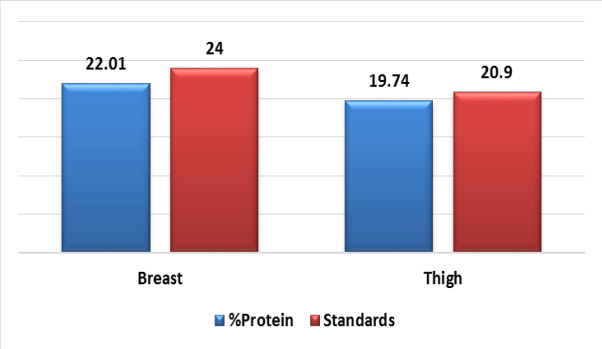


**Figure 1. Crude protein in two different parts of the chicken meat sample. Standard according to (Agency, 2002)**

**Results of protein percentage in fresh and frozen veal meat (Silverside).**

The results in Fig. 2. are protein content in frozen and fresh silversides of veal meat, showing higher protein content (20.55 %) in fresh meat while the lowest value (19.08 %) was recorded in frozen. The results of protein content determination by Kjeldahl method in fresh and frozen silverside veal meat are lower than those recorded by Omotayo *et al*. (2016), perhaps due to using different types of meat, which is cow meat, additionally proteins in meat vary according to factors such as the age of animal.

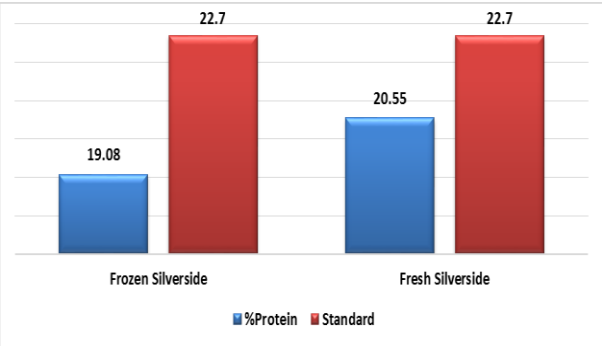


**Figure 2. Crude protein in fresh and frozen Veal meat samples. Standard according to (Agency, 2002)**

**Results of crude protein in egg samples.**

The protein content of egg-white and egg-yolk in Turkish egg samples are illustrated in fig. 3. The results indicate a non-significant difference between standard with the Kjeldahl method in both parts of the egg.. Clear indication of reproducibility of the Kjeldhal method when good laboratory practice are followed.

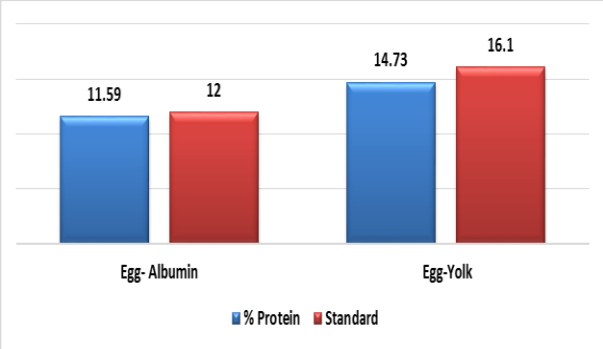


**Figure 3. Crude protein in egg-white and egg-yolk in Turkish egg samples. Standard according to (Coultate, 2002).**

**Results of crude protein in beef hot dog sample.**

The protein content of beef hot dog is shown in fig. 4. The results indicate to non-significant difference between the declared value of protein (13.00) with the Kjeldahl method in beef hot dog samples. The result of the Kjeldahl method for determining protein in beef hot dog samples is lower than the protein content reported by Mihaljev *et al*. (2015),which might be due to using a different meat content in the hot dog brand used.. Whereas the results of protein content by Kjeldahl method used in this work shows lower than those recorded by Anderson (2007), this may be due to using a different type of hot dog emulsion through the variation in the specific amino acid composition of proteins. Proteins rich in basic amino acids contain more nitrogen than those missing in basic amino acids.

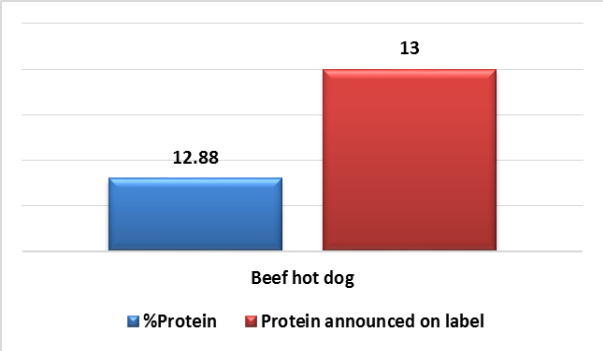


**Figure 4. Crude protein in beef hot dog sample**

## CONCLUSION

Based on the analysis of protein values for the product samples obtained in this work, it can be stated that chicken breasts contain the highest percentage of crude protein followed by fresh silverside, fresh chicken thigh, frozen silverside, egg-yolks, beef hot dog, and lastly eggwhites. Also we concluded that the validity of the claim of protein content declared on the can of the beef hot dog, as well the results of eggalbumin and fresh chicken thigh were found not significantly different ($P \leq 0.01$ ) to the standard. Upon these results obtained for protein contents in selected animal products (chicken, veal meat, egg, beef hot dog), the fresh chicken breast is recommended for human consumption compared to the other protein sources . Also, people on diet of low protein intake could take protein in sufficient amount on daily basis from animal or plant sources.

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## تقدير البروتين في بعض المنتجات الحيوانية من أسواق السليمانية باستخدام طريقة كلدال


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في هذه الدراسة ، تم قياس البروتين الخام لعينات المنتجات الحيوانية من خلال تحديد محتوى النيتروجين الكلي باستخدام طريقة كلدال. يعد قياس إجمالي النيتروجين بواسطة كلدال هو الأسلوب المرجعي العالمي لتقدير محتوى البروتين في المنتجات الحيوانية والنباتية ويستخدم في كل من المعايرة والتحقق من صحة الطرق البديلة في تقدير البروتين. مصادر البروتين الحيواني التي تم تحليلها في هذه الدراسة كانت عينات من لحم العجل ولحم الدجاج والبيض والنقانق. بناءً على النتائج التى تم الوصول اليها في هذا البحث ، كان تسلسل محتوى البروتين في العينات هو: صدور الدجاج > ظهر الفخذ الطازج > فخذ الدجاج الطازج > ظهر الفخذة المجمدة > صفار البيض > نقانق اللحم بقري > بياض البيض. وتطابقت نسبة البروتين مع النسبة المثبتة على علبة نقانق اللحم البقري ، اما نتائج بياض البيض و فخذ الدجاج الطازج، لم تظهر فروق معنوية مقارنة بالحدود القياسية.