# Determination of Optimal Protein Amount in Macedonian White Brined Cheese

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DOI: https://doi.org/10.52403/ijrr.20230737

#### ABSTRACT

White brined cheese is a popular dairy product that is enjoyed by people all over the world. One of the key nutrients found in white cheese is protein, which plays a crucial role in maintaining the health and function of the human body. This protein is an essential nutrient that is required by the body for a range of functions, including building and repairing tissues, supporting immune function, and regulating hormone production. In this research paper the optimal protein amounts for Macedonian white brined cheese were determined. White brined cheese is Macedonian traditional product with specific taste and quality parameters. Four variants of Macedonian white brined cheese were analysed for their protein amount during ripening period of 60 days. The protein amount of white brined cheese comes primarily from casein, which is a slow-digesting protein found in milk. Casein is considered a high-quality protein, as it contains all the essential amino acids required by the body to build and repair tissues. According to the data obtained from the laboratory analyses, it was determined that the optimal range for protein amount in Macedonian white brine cheese was between 12.91±0.01% to 13.18±0.01%. Also it was determined that the protein level in analysed cheese samples were statistically significant at level p<0.05;

*Keywords:* white brined cheese, protein, casein, optimal amount;

#### **INTRODUCTION**

The white-brined cheese is our traditional high-quality product, that is very popular in the Balkans and in all East Mediterranean countries. It is a nutritious and delicious food that provides a good source of high-quality protein, along with a range of other essential nutrients. This type of cheese has a high nutritional value, due to high content of total protein (17-18%) and fat (22-25%). It is also rich with minerals and vitamins (A and D), (Beev et al., 2019).

White cheese is a good source of protein, which is an essential nutrient for the body. According to the United States Department of Agriculture (USDA), 100 grams of white brined cheese contains approximately 14 grams of protein. This makes it a good source of protein, this is a significant amount, as the recommended daily intake of protein for an adult is approximately 0.8 grams per kilogram of body weight (Rodriguez et al., 2009).

Protein in white brined cheese comes primarily from casein, which is a slowdigesting protein found in milk. Casein is considered a high-quality protein, as it contains all the essential amino acids required by the human body to build and repair tissues.

Caseins are the main protein in cheese, which exist in the form of aggregates after combination with colloidal calcium phosphate known as casein micelles. Caseins in cheese are of nutritionally rich due to the high supply of essential amino acids, phosphate and calcium, (Farrell et al., 2004). The fermentation process of white brined cheese has been done in brine solution and also in anaerobic conditions. Acid-salty flavor, no rind, usually white color, occasionally with yellowish tint are the main characteristics of this type of cheese, (Veleski, 2015). White brined cheese and its ripening are dynamic systems that are chemically, microbiologically and enzymatically complex, (Mallatou et al., 2004).

Physical, chemical, biochemical, microbiological and sensorial changes occur during the ripening period. Proteolysis is the principal and most complex biochemical reaction that occurred during the ripening process of most cheese varieties. Proteolysis, like in most cheese varieties, has a significant role in the development of cheese characteristics, (Fox, 1989).

The aim of this scientific research is to follow the total protein amount of Macedonian white brined cheese during ripening period and to determine the optimal amount for this significant parameter.

## LITERATURE REVIEW

The biochemical reaction is the most significant process that indicates the ripening of white brine cheese. This reaction occurs as a result of the hydrolysis of casein under the action of enzymes (Fox et al., 2000). The result of hydrolysis is the breakdown of proteins into low molecular weight peptides and amino acids. Proteolysis in cheeses is monitored through the change of total nitrogen and total nitrogen fractions. During ripening, the proteins that are part of the structure of the cheese are broken down to polypeptides and amino acids, which affect the formation of the taste and aroma of the cheese itself (Fox et al., 1993). The average value for this qualitative parameter in white brine cheese, according to the data obtained by a large number of authors, ranges in the following limits: Naydenova et al., (2013) -14,20% to 14,65 %, Smiljanic et al., (2014)-13,62%, Ivanov et al.,  $(2016) - 14,4\pm0,4\%$ , Balabanova et al., (2017) - 14,4±0,3% and etc.

# **MATERIALS & METHODS**

In order to make these examinations, cheese samples of four variants (WBC 104, WBC 105, WBC 110 and WBC 111) were provided from the producers, and were transported in the Certified Laboratory for testing milk and dairy product quality-LB Lact in Plovdiv, R. Bulgaria. Cheese samples for total protein content (%) were analyzed at 8th, 20th, 30th, 40th and 60th day during fermentation. The laboratory method that was used for determination the protein content of white brined cheese was as follow:

In order to calculate the protein content in milk products, it is necessary to determine the nitrogen content in the analysed sample, and this is achieved in three successive procedures: digestion, distillation and titration.

For analysis, it is necessary to weigh 10 g cheese sample on analytical scale. Digestion was performed in special Kjeldahl test tubes, in which the cheese sample was placed together with all reagents (Concentrated Sulphuric acid, Kjeldahl tablets). The digestion takes place at a temperature of 420°C, in a period of 30 minutes. After digestion process, the sample must be cooled down to a temperature of 50-60oC. For this experiment Velp DKL8 digestion unit was used.

The next process of this experiment was distillation that was done by using the distillation unit model Velp UDK149. The following reagents for each distillation were used: (distillate water (50 ml), boric acid (4% H3BO3) (25 ml) and sodium hydroxide (40 % NaOH) (50 ml)).

After distillation process the final part of the experiment was titration in order to get the nitrogen content of the analysed cheese sample. The distillate was titrated by using 0,1 M HCl. The amount of 0,1 M HCl used for titration is needed to determine nitrogen content in analysed cheese sample.

$$N \% = \frac{1.4007 x (V \text{ sample} - V \text{ blind sample}) \times Mt}{W}$$

- Protein factor 1.4007;
- V sample mL 0.1 M HCl used for titration process of cheese sample;
- V blind sample mL 0.1 M HCl used for titration process of blind sample;

- Mt-molarity of HCl x factor of HCL used for titration;
- W-cheese sample mass (g);
- Protein (%) = N% x 6.38;

# **Statistical Analysis**

Statistical processing of the obtained results was interpreted by using variation-statistical methods, which are mostly applied for this kind of scientific research.

For data processing Microsoft Excel programme was used, which is part of Microsoft office package. With its use the data were tabular and graphically represented, the parameters average mean (x), coefficient of variations (CV) and standard deviation (SD) were calculated. By using t-test, that is also part of this package, statistical significant comparison between each quality parameters of the variants were made.

## RESULT

Comparative analysis of protein dynamics between experimented white brined cheese variants is presented in Table 1.

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Protein (%)					
Day	WBC 104	WBC 105	WBC 110	WBC 111	
8	13.20±0.02ª	12.80±0.03b	13.40±0.02°	13.62±0.10 <sup>d</sup>	
20	13.40±0.02 <sup>a</sup>	13.21±0.03 <sup>b</sup>	13.54±0.01°	13.59±0.03 <sup>c,d</sup>	
30	13.61±0.01 <sup>a</sup>	13.51±0.01 <sup>b</sup>	13.95±0.02°	13.93±0.02 <sup>c,d</sup>	
40	13.07±0.04ª	13.05±0.02 <sup>a,b</sup>	13.25±0.03°	13.32±0.01 <sup>d</sup>	
60	12.93±0.01ª	12.91±0.01b	13.02±0.01°	13.18±0.01 <sup>d</sup>	

\*Differences of values with different superscripts in the same row are statistically significant at level p<0.05;

According to the results presented in Table 1, at the end of fermentation process the protein amount was between 12.91±0.01% to 13.18±0.01%. The highest amount of protein is noticed at variant WBC 111, which is 0.16%, 0.25% and 0.27% higher than other examined variants WBC KS110, WBC KS104 and WBC KS105, respectively. From Table 1 it can be concluded that there are significant differences in protein amount in all four cheese variants, at level p<0.05, in most of examined periods, and that can be explained with different technology and conditions during white brined cheese production process.

During the ripening period (day 8-th) cheese variant - WBC 111 had the highest percentage of protein amount-13.62±0.10% compared to the other variants. The lowest protein percentage was noticed in cheese variant - WBC 105 - 12.80±0.03%.

After 20 days ripening, the protein amount in all examined variants was ranged within the following limits: 13.21±0.03% (Variant WBC 105) to 13.59±0.04% (Variant WBC 111).

In further period of the ripening process on the 30th day, the highest protein amount was observed in all examined cheese samples, and they ranged in the following limits:  $13.51\pm0.02\%$  to  $13.95\pm0.03\%$ . After this approach, it was normal to expect a decrease in protein amount, because the process of active breakdown of proteins begins, accompanied by a significant release of free amino acids and volatile components, carbon dioxide and nitrogen).

On the 40th day, a decrease in the protein amount of the four variants was observed in comparison to the previously examined period, and it ranged from  $13.05\pm0.03\%$  to  $13.32\pm0.01\%$ .

The protein amount on the 60th day in the examined cheese samples reached amount of  $12.91\pm0.01\%$  to  $13.18\pm0.01\%$ . From the processed data of protein amount, it can be concluded that the variant WBC 111 has the highest percentage of proteins, which is 0.16%, 0.25% and 0.27% higher than the other variants WBC 110, WBC 104 and WBC 105, respectively, which we consider that is the result of the activity of starter cultures added to the milk.

From Table No. 1 it can also be concluded that there is a significant difference in the protein amount between the four varieties of white brine cheese at the p<0.05 level, and that was noticed in most of the examined period. The application of different technology and conditions during the production process of white brined cheese are the two main reasons for the significant differences between analyzed cheese samples.



# DISCUSSION

Approximate protein amount with our results, were also obtained by Smiljanic et al., (2014)-13.62%, as well as by Lavasani (2014), which examined the effect of different concentrations of rennet ferment during the process of production of white brine cheese, and have determined amount for this parameter of 13.66-13.96%.

Minimally higher protein amount compared to our obtained results were presented by Balabanova et al., (2017) which have determined an average total protein amount of 14.4  $\pm$ 0.3% in Bulgarian white brined cheese. Ivanov et al., (2016) also have determined amount of 14.4 $\pm$ 0.4% total protein after 45 days of the ripening period. Similar to previous authors, the total protein amount obtained by Felfoul et al., (2016) -14.14%, as well as Naydenova et al., (2013) which determined a total average protein amount of 14.20-14.65%.

# CONCLUSION

According to the data obtained from the laboratory analyses in our research, it was determined that the optimal range for protein amount in Macedonian white brine cheese was between  $12.91\pm0.01\%$  to  $13.18\pm0.01\%$ . Also it was determined that the protein level in analyzed cheese samples were statistically significant at level p<0.05; From our results

and the results obtained by other researchers it can be concluded that optimal value for protein content in white brined cheese must be within the range from 13% to 18%. This optimal value was achieved in the production process of all four variants white brined cheese.

Declaration by Authors Acknowledgement: None Source of Funding: None Conflict of Interest: The authors declare no conflict of interest.

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How to cite this article: Makarijoski Borche. Determination of optimal protein amount in Macedonian white brined cheese. *International Journal of Research and Review*. 2023; 10(7): 283-287.

DOI: https://doi.org/10.52403/ijrr.20230737

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