

TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

Comparison of protein amount of same kind and same amount of skimmed pasteurized milk, which are heated under different wavelength power.

Biology Extended Essay

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ABSTRACT

Heating milk is a duration, which is usually used for processing of several dairy products. In today's fast lives use of microwave is become widespread for saving time. A major consequence of the heat treatment of milk is the damages of the proteins. The microwave effect on proteins, which are found dairy products, cannot be clarified yet. The purpose of this experiment is to investigate the amount of totally proteins in which is exposed to different microwave electrical power.

This studies question was:“ how does the quantity of proteins in pasteurized milk change when it is heated in a microwave oven, for different wavelengths.

It was hypothesized that; a significant mean difference would occur, in the amount of proteins of milk in which was exposed to different wavelengths microwave.

In order to examine the hypothesis and answer the research question, 25 samples of 5 ml milk were divided 5 groups. Groups were exposed in unclosed vessels to the microwaves action for each of the following 5 different wavelengths during 30 second. The Pierce BCA protein Assay was used to measure the protein amount. This method is a detergent – compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein.

The results showed that the averages of protein increased during the microwave exposure. ANOVA results revealed that there was a significant mean difference among the groups. We expected that the protein amount of microwaved milk would decrease depending upon the denaturation of protein. Contrary to expectation, protein amount in milk is increased in our investigation. This amazing result can be explained by evaporation during the microwave exposure.

These results indicate that the total amount of protein in milk does not affected during microwave heating.

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TABLE OF CONTENTS

I. Introduction	4
II. Hypothesis	7
III. Method development and planning	8
IV. Method	13
V. Results	14
VI. Data Analysis	16
VII. Evaluation	19
VIII. Conclusion	22
IX. Appendices	25

I. INTRODUCTION

This extended essay analyses the effects of microwaves on proteins that attend in milk. I preferred to focus on this subject, because of my observations, which are based on society. Recently, I observed that the microwaves ovens are used a lot almost in every house. I had been told that the microwave ovens have more serious negative effects on human beings. As an article indicates, a microwave oven is a device, which is used for cooking or reheating food in a faster way than conventional oven. It does this process by heating the water and particles within the food faster. In this way frozen food can turn into hot meals by using microwave technology. Many people still believe there are some health risks associated with using this type of oven for cooking although the technology behind microwave ovens has been around for nearly seventy years.¹The most significant example that proved this consideration is blood products. Microwaved blood products damages the blood components.² This information clearly shows that the microwaves damage users' lives.

It was sounded very interesting to me, and I began researching this issue. I found that, fast-paced modern life cannot be separated from the microwave, especially breakfast, when the pursuit of more speed and convenience, while the heating of milk has become the most common phenomenon. A microwave oven is a kitchen appliance which uses microwave radiation to excite polarized molecules of food, and provides food to heat in an incredibly fast.

As an Australian study has proved, microwaves cause a higher degree of "protein unfolding" than conventional heating. Microwaving can destroy the essential disease-fighting agents in breast milk that offer protection for a baby.³ In 1992, Quan showed that microwaved breast milk lost lysozyme activity, antibodies, and fostered the growth of more potentially pathogenic bacteria. Quan indicated that more damage was done to the milk by heating it by using microwave method than by other different methods of heating, concluding: "Microwaving appears to be contraindicated at high-temperatures, and questions regarding its safety exist even at low temperatures."

¹ <http://www.wisageek.com/what-is-a-microwave-oven.htm>

² <http://www.truthistreason.net/ten-reasons-to-throw-out-your-microwave-oven>

³Quan R (et al). Effects of microwave radiation on anti- infective factors in human milk. Pediatrics 89(4 part I). 667-669.

To consider the use of microwave to heat milk will destroy the nutritional elements, this problem lies in process of heating. Because the excessively fast microwave heating, the milk nutrients cannot be conserved, under high temperatures.

According to the information, the microwave disinfection predominantly provides by the thermal effects and biological effects. Thermal effect of bacterial cells can heat denatured protein coagulation, which is resulting in the death of bacteria.⁴ Using microwave to heat milk for high wavelength, makes the protein in milk by high-temperature role, by the sol-gel state into a state, resulting in the sediment, thereby affecting the quality of dairy products. Heating milk under higher the temperature, in which the more serious loss of nutrients, and major vitamins, followed by lactose.

After I had read the information, which is about disappearing of proteins in milk after microwaved, and because of the amount of protein that it has I decided on launching my investigation on microwave effects on milk. Heating milk in a microwave oven is a process, which is done frequently in daily lives of people. For example, it is needed to heat milk fast and untimely, in baby caring part of our lives, parents usually use microwave ovens to heat milk faster but they do not know how it could be harmful for health of their child. Children's defence systems do not work formless since they do not complete their development properly. According to this, giving microwaved milk as a feed to a children is more dangerous than giving it to an adult. Consequently, I chose milk as a material to use in this investigation because it could be more harmful than other nutrition for a human life.

Protein content can also decrease during the microwave exposure due to the fact that protein can be involved in the Maillard reaction together with the lactose. Among the proteins, the lysine is the most implicated in this reaction and its diminution in time became a nutritional indicator for the appreciation of the heat treatments on food⁵

This study is conducted to determine the research question: How does the quantity of proteins in pasteurized milk change when it is heated in a microwave oven, for different wavelengths. It is used different wavelengths in this investigation to determine the harm that caused by intensity of wavelength. Also I chose pasteurized

⁴ http://www.love-health.org/diet_food/1144/

⁵T. Florea, *Chimia alimentelor*, vol 2 ed. Academica, Galati, 2001, 360.

milk as a material to use in this research since the pasteurized milk is the most common milk type that is used in daily lives of humans as nutrition. In addition, it will discuss how the investigation planned and performed and analyse the possible consequences of this process.

II.HYPOTHESIS

As a result of committed experiments it is thought that microwaves affect food in which there are organic molecules.

A Japanese study by Watanabe proved that just 6 minutes of microwave heating turned 30-40 per cent of the vitamin B12 in milk into a non-effective (dead) form. This study has been cited by Dr. Andrew Weil as an evidence that supporting his concerns about the effects of microwaving. Dr. Weil⁶ told that there might be hazards associated with microwaving nutrition. Furthermore there is a question as microwaving method changes chemical structure of the protein in ways, which could be harmful.

An article, which was published in 2006 by Kaddourı Hanane claim that microwave, causes more conversions in whey proteins composition. Whey proteins are the most valuable proteins in milk. Milk proteins are casein %80 and Whey protein %20. Microwave heating of cow's milk appears to decrease its whey proteins reactivity against the specific proteins (Ig G).⁷

In light of the information above, it is determined that there would be a considerable mean difference in amount of protein, in same volume of pasteurized milk, which is heated, in different wavelengths, in proper conditions.

According to information above, it is hypothesized that there would be a significant mean difference proportional to efficiency change of wavelength.

⁶ <http://canadianawareness.org/2012/07/microwaving-food-is-unhealthy/>

⁷ African journal of biotechnology Vol.5 (13), pp. 1267-1270, 3 July 2006

III. METHOD DEVELOPMENT AND PLANNING

This investigation is planned to present the effects of the process, that heating milk under different intensities of microwave, on proteins of pasteurized milk. It was made in department of biochemistry, in Hacettepe University Faculty of Pharmacy. One of the Assistant professors who worked there helped me when the study was performed. She showed me the use of laboratory equipment, and helped about the performing of the technique that we had chosen and making measurements.

After a short definition of bovine milk proteins, several methods of current or potential use for specifying them in dairy products reviewed.

Methods that allow resolution of some milk protein fractions (casein, whey proteins, β -lacto globulin) include, the Aschaffenburg-Rowland procedure, dye-binding and infrared methodologies. Different methods (electrophoresis, column chromatography, immunochemical or enzymatic tests) can use for determining and individually quantitating the diverse caseins and whey proteins.⁸

In this investigation, amount of total protein in milk was investigated.

The methods that have been used for analysing totally milk protein can be divided into three categories:

a: Nitrogen analysis with subsequent arithmetical calculation of the protein content using conversion factors,

b: Protein analysis using colorimetric methods (such as the Lowry method) or spectroscopic methods (such as infrared spectroscopy),

c: Amino acid analysis⁹

Currently there are different methods used for resolution of total protein in milk.¹⁰ Because, in general, these methods are not standardized against a reference method, values stated in the literature values sometimes diversify regularly. Furthermore, no

⁸<http://www.cabdirect.org/abstracts/19930457633.html>

⁹Lonnerdal B, Smith C, Keen CL. Analysis of breast milk: current methodologies and future needs. J Pediatr Gastr Nutr 1984;3;290-5.

¹⁰ Lonnerdal B, Smith C, Keen CL. Analysis of breast milk: current methodologies and future needs. J Pediatr Gastr Nutr 1984;3;290-5.

systematic comparison of various methods for analyse to total protein of milk has developed, despite the Lowry method¹¹ or a modification theory¹² has been suggested by at least two laboratories¹³.

The Pierce BCA protein Assay used in this experiment because of its high accuracy (see appendices I). There are several reasons that make me choose this method. Primary one is, this method would procure me with quantitative data for statistical analysis of the investigation. Another reason is, the method is usually used by searchers, because of its high precision and acceptable constant and proportional error.^{14,15}

I have chosen the BCA method because Ronald P claimed that this method has the least difference in values among different types of protein. This method has the greatest accuracy with acceptable constant and proportional error.

For colorimetric analysis it is excessively substantial to defeat the samples completely to refuse the light-scattering effects of the milk fat spots. That is why I have obtained the raw cow milk, which is pasteurized, from the local market in which consists of no fat.

In order to see what effects microwaves have on its amount of totally protein, 25 samples of 5 ml milk were divided 5 groups. The volume of pasteurized milk in every trial is the constant variable of this investigation. It is stabilized by measuring with a graduated cylinder before it is used in the experiment. Moreover, this investigation contains 5 trials for five different wavelengths; it means that in total, there are 25 trials in this experiment. Groups were exposed in unclosed vessels to the microwaves action for each of the following 5 different wavelengths during 30second:

W₉₀ - samples is treated with microwaves at electrical power of 90 W,

¹¹ Lowry OH, Rosebrough NJ, Farr AL, Randall LJ. Protein measurement with the Folin phenol reagent. J Boil Chem 1951;193:265-75.

¹² Peterson GL. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal Biochem 1977;83:346-56.

¹³ Patton S, Huston GE. A procedure for the determination of total protein in human milk. Nutr Rep Int 1984;30:1401-8. / Lonnerdal B, Forsum E, Hambraeus L. The protein content of human milk. I. A transversal study of Swedish normal material. Nutr Rep Int 1976;13:125-34.

¹⁴ Ronald P. Keller and Margaret C. Neville. Determination of total protein in human milk: comparison of methods. Clin. Chem. 32/1, 120-123 (1986).

¹⁵ www.protein.ege.edu.tr/Konular/Protein%20tay.pdf

W_{180} - samples is treated with microwaves at electrical power of 180 W,

W_{300} - samples is treated with microwaves at electrical power of 300 W,

W_{600} - samples is treated with microwaves at electrical power of 600 W,

W_{900} - milk samples is treated with microwaves at electrical power of 900 W, microwave heating was carried out using a Gaggenau BM 220/221 microwave oven.

During this experiment we came up against with several problems. First of all I had some difficulties while using the laboratory equipment so that the official Mrs Özden helped me with using the equipment. When we started to experiment we saw that the microwave oven in the laboratory worked with only for 4 different wavelengths, so that we could not use it and we carry a usual microwave oven to laboratory to do the experiment as it was planned. After that when we were heating the milk, the milk poured out of the test tubes that we used in the experiment so that we had to replace test tubes with beakers.

Because of the BCA protein indication techniques require a standard studying solution of reactive A and reactive B; reactive A and B are prepared first. For preparing reactive A; 10g BCA, 20 g Na_2CO_3 , $\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$, 1-6g $\text{Na}_2\text{C}_4\text{H}_4\text{O}_6$, H_2O , 4g NaOH, 9,5g NaHCO_3 are mixed with 1 liter distilled water. For preparing reactive B; 2g CuSO_4 is mixed with 50 ml of distilled water. Materials used for preparing reactive solutions are listed in appendix 2.

Reactive A and reactive B can be stable for 12 months in room conditions.

After preparing reactive A and reactive B, 50ml of reactive A and 10ml of reactive B are mixed to prepare standard reactive solution. This standard reactive solution can be used for one week.

0.1ml milk is taken from every samples of milk before and after it is microwaved at different electrical power (totally 50 samples) and then mix with 2ml standard reactive. They have been waited at room temperature for 30 minutes. Milk sample contain a comparatively large concentration of protein and hence need to be anticipated only briefly 30 minute in the protocol.

The samples are then analysed spectrophotometrically with the device called Micro plate Carrier Shipping Bracket Removal Instruction (BioTek Power Wave XS). The concentrations of protein in microgram/ml were determined. The results were then statistically processed in Microsoft office Excel using ANOVA.

Materials used in the experiment:

- 125 ml pasteurized skimmed milk
- Gaggenau BM 220/221 microwave oven
- Microplate Carrier Shipping Bracket Removal Instruction (BioTek Power Wave XS)
- Graduated cylinder
- 5 identical wide beakers

For preparing reactive A and reactive B solutions:

- 10 g BCA,
- 20 g $\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$
- 1.6 g $\text{Na}_2 \text{C}_4 \text{H}_4\text{O}_6 \cdot \text{H}_2 \text{O}$
- 9.5g NaHCO_3
- 4g NaOH
- 2g CuSO_4
- 1050 ml distilled water

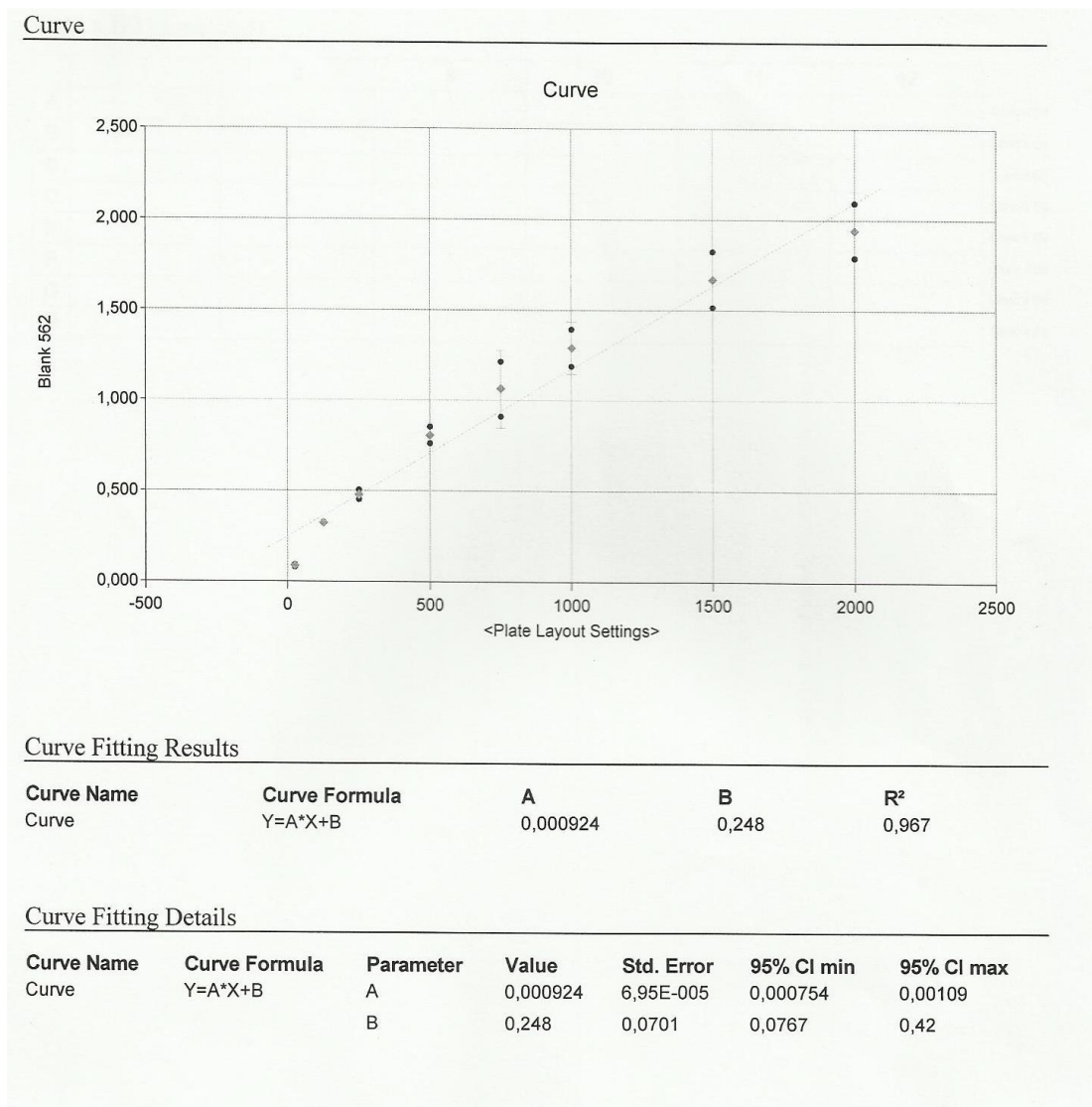
IV. METHOD

Procedure:

- Reactive A and reactive B prepared
- Standard reactive prepared by mixing 50 ml of reactive A and 10 ml of reactive B are mixed.
- 5 ml of pasteurized milk is measured by graduated cylinder and it is heated under different wavelengths (90W, 180W, 300W, 600W, 900W). This process is repeated five times.
- 0.1 ml milk is set apart after it is heated and mixed with 2 ml standard reactive solution. This process is repeated for all 25 trials.
- Solutions, which contain milk, and standard reactive solution are waited for 30 minutes under room conditions.
- Absorbance measurement is done under 562 nm in spectrophotometer, for all trials.
- Prepare a standard curve by plotting the average blank-corrected 562 nm measurements for each BCA standard vs. its concentration.
- To determine the protein concentration of each unknown sample, use the standard curve.

V. RESULTS

Figure 1, shows the values for the individual colorimetric reactions, plotted as a function of the BCA protein values.



This standard curve was produced by plotting the average blank-corrected 562 nm measurements, for each BCA standard concentration. This standard curve was used to specify the protein concentration of each unknown sample by the device called Micro plate Carrier Shipping Bracket Removal Instruction (BioTek Power Wave XS).

The table below represents the initial value of protein amount and the final value of protein amount.

microwaves power used (W)(±0.1)	trials	type of milk used	volume of milk used (ml) (±0.5)	time (sec) (±0.05)	Initial value of protein amount (µq/ml)	Final value of protein amount (µq/ml)
90.0	1	pasteurized milk	5.0	30.00	25,000,574	25,476,986
	2				25,001,205	25,478,186
	3				25,041,005	25,516,405
	4				25,079,102	25,557,402
	5				24,949,975	25,424,975
180.0	1	pasteurized milk	5.0	30.00	25,020,105	25,587,194
	2				25,036,002	25,621,199
	3				25,029,029	25,614,236
	4				24,989,895	25,582,035
	5				24,968,973	25,577,013
300.0	1	pasteurized milk	5.0	30.00	25,032,375	25,780,157
	2				25,016,268	25,766,243
	3				24,989,987	25,771,006
	4				24,917,996	25,698,614
	5				25,078,672	25,858,598
600.0	1	pasteurized milk	5.0	30.00	24,899,956	26,386,500
	2				25,019,205	26,404,795
	3				24,998,876	26,380,861
	4				25,037,452	26,426,506
	5				25,010,205	26,392,253
900.0	1	pasteurized milk	5.0	30.00	25.091.205	26.298.950
	2				24.992.100	25.987.902
	3				25.030.572	26.117.850
	4				25.118.005	26.380.250
	5				24.950.978	26.750.115

Table 1: different microwave powers effects on proteins, which belong to 5ml-pasteurized milk under certain conditions

VI. DATA ANALYSIS

Trials	W90	W180	W300	W600	W900
1	476.412	567.089	747.782	1486.544	1307.745
2	476.981	585.197	749.975	1385.590	1395.802
3	475.400	585.207	781.019	1381.985	1787.278
4	478.300	592.140	780.618	1389.054	1862.245
5	475.000	608.040	779.926	1382.048	1999.137

Table 2: table that shows the difference in protein values between non - microwaved and microwaved pasteurized milk.

Mean	476.4186	587.5346	767.864	1405.0442	1670.4414
Mode	#N/A	#N/A	#N/A	#N/A	#N/A
Median	476.412	585.207	779.926	1385.590	1787.278
Range	3.300	40.951	33.237	104.559	691.392
Variance	1.7264938	217.6464483	301.1283575	2084.230428	91363.92203
SD	1	15	17	46	302
SE	1	7	8	20	135
T	2.776445105	2.776445105	2.776445105	2.776445105	2.776445105
%95CI(SE X T)	1.631498206	18.31807704	21.54665792	56.68614913	375.3111408
%95CI(EXC EL)	0.814386569	9.14374031	10.75533442	28.29573353	187.342132

Table 3: table of descriptive statistics

H₀:heating pasteurized milk in different microwave power does not affect the volume of proteins, which attend in pasteurized milk.

H₁: heating pasteurized milk in different microwave lengths affects the amount of total protein, which attends in milk.

SUMMARY

Groups	Count	Sum	Average	Variance
W90	5	2382.093	476.4186	1.7264938
W180	5	2937.673	587.5346	217.6464483
W300	5	3839.32	767.864	301.1283575
W600	5	7025.221	1405.0442	2084.230428
W900	5	8352.207	1670.4414	91363.92203

ANOVA

source of variation	SS	Df	MS	F	P-value	F crit
between groups	5549931.159	4	1387482.79	73.82689515	1.09283E-11	2.866081
within groups	375874.615	20	18793.73075			
total	5925805.774	24				

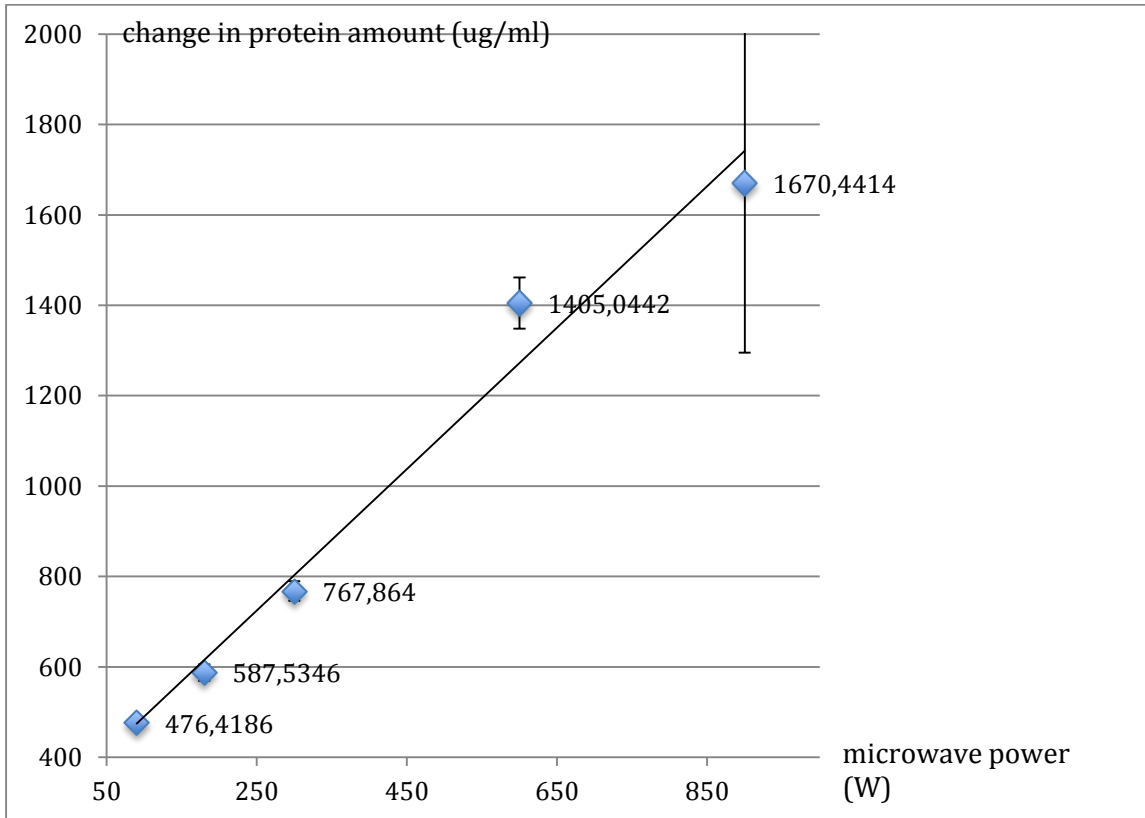


Chart II: Graph of microwave power effects on milk protein

According to the Anova results there is differences, which can be accepted as significant, between the groups. As it can be seen from the graph, the more power of microwave increases, the more differences between groups and the height of the error bars increases. This case shows that possibility of error increases when the wavelength of microwave increases, during the experiment.

VII. EVALUATION

After reviewed the various methods of current use for detecting bovine milk proteins this study was planned. The main purpose of this investigation is to show whether there was a mean difference in the amount of proteins in pasteurized skimmed milk which microwaved under different microwave powers when the amount of milk heated is constant. Heating food can result in some nutrients loss. It was predicted that a significant mean difference would occur, in the amount of totally protein, which attends in milk by heating it (pasteurized milk) in different microwave lengths.

The difference of protein amount between the non microwaved and microwaved milk, specifically increased in milk groups which had been heated under high wavelengths. In the group which had heated under 90W, mean difference in protein amount is 476.42 when in the group which had heated under 900W, mean difference in protein amount is 1670.45.

My first hypothesis defended on there was not any difference between non-microwaved and microwaved milk in their protein amount. p values of pair-wise comparisons of the groups were found to be smaller than 0.05, that's why the first hypothesis was declined. There is an apparent mean difference between the groups in terms of protein values (see table 4).

The coefficient of variation and the standard deviation are applied to the obtained averages of the data. There were some differences between the groups. The averages of protein amount increased during the microwave exposure. Amount of protein in pasteurized milk increased in all of the groups after microwaved exposure (Table 1).

I did not expect that rise in protein value of microwaved milk. It appears that my estimations of the microwaved effect on milk were accelerated.

All of the groups have significant differences in the amount of protein values. The difference of protein value is clearly maximum in the milk samples, which is treated with microwaves at electrical power of 900 w.

Standard deviation values of groups are regularly increased. It was 1 in group 90w, 15 in group 180w, 17 in group 300w, 46 in group 600w and 302 in group 900 w.

Along the study there were not any unpredicted circumstances, which might have changed the outcome data of the investigation. Nevertheless while writing the essay; I noticed some achievable alternative tenders in the method, which might have effects on the results.

1. We investigated the difference of totally protein amount in microwaved pasteurized milk, and after microwaved effect, totally protein amount was increased. If we evaluated the specific proteins in milk (such as casein, globulin, albumin) we would have found some diminish in values.
2. Microwave ovens do not heat food equally. In other words same parts are overcooked, that can change their molecular structure into dangerous substance while other parts are undercooked increasing the risk of foodborne disease.¹⁶ According to this, amount of protein can be increase or stay stable, but it doesn't show that using microwave doesn't damage milk proteins.
3. Evaporation effect on protein amount did not consider when the experiment was planned. It is thought that as a result of evaporation protein amount of milk might increase relatively. Preliminary studies show that removing the water concentrates the nutrients so evaporated milk has more nutrients¹⁷. If a mechanism, which could be prevent the evaporation, was set up or density of samples could be measured besides measuring the amount of protein, it could be shown that the increasing in the amount of protein is because of increase in density.
4. The temperature in microwave-treated foods depends on the type and quantities of this food. The milk boiled at the surface before the temperature increases. When the boiling begins at the surface, it hasn't started at the bottom and in the centre yet. The temperature at the surface was evidently higher than the bottom, middle or side of the flasks.¹⁷ It is not considered there was boiling or not in the milk which was heated under low

¹⁶ www.globalresearch.ca/the-dangers

¹⁷ www.agroscope.Admin.ch/data_publication/1315408248-316.pdf

microwaves. Besides, the milk, which was mixed with reactives, wasn't taken from specific parts of the beaker (top, middle, bottom). In some measurements, milk might be used before it isn't heated enough.

VIII. CONCLUSION

The aim of this research is answering the question: "Is there a significant mean difference between same kind and volume of pasteurized skimmed milk samples when they are heated under different wavelength powers in a microwave oven?" My research question is answered in the direction of the results of the investigation. There is a difference in protein amounts of the groups in which influence of different wavelengths. There is a little change occur between the protein amount before and after heating. Amazingly the protein amount is increasing. In spite of the method can be modified for more measuring instruments, I was persuaded the result of the study that explain the microwave effect on totally protein amount of milk.

The reason I preferred to focus on this subject, for my extended essay, is the prevalence of microwave ovens in houses, although there are many reports about negative effects of microwave ovens. However this is a subject that must be limited to make an investigation on it because there are much nutrition types that can be observed under microwave power, in environment. So, I decided to restrict my investigation as the microwaves affects on same volume of pasteurized skimmed milk, under different wavelengths. There are some extensive research that conducted the accurate methods on this subject, but there is no study that I have detected which compares the total protein amounts of equal volumes of skimmed pasteurized milk, which are heated under different microwave wavelengths. I have chosen to write my essay on this subject because it has not been tried before.

In the recent years, a study has been published in which the influence of microwave heating on the isomerisation and racemisation of amino acids, have been explained. Studies applied on whole milk were conducted by Petrucelli and fisher¹⁸. Whole milk samples were heated for ten minutes in a microwave oven at medium power. In this investigation, they showed that heating milk in microwave under normal domestic provisions does not generate mean changes in amino acids of their protein. The energy of microwave radiation is too little to break chemical bonds of the proteins.

¹⁸Anka Dumuta, Physical and chemical characteristics of milk variation due to microwave radiation. Croatica chemica acta84(3)(2011)429-433.

Although, Quan claimed that microwaving is contraindicated at high-temperatures, and trouble considering its safety subsist even at low temperatures; we could not show this effect. Structure of protein may be changed but our study did not investigated this issue. Amount totally protein did not diminish.

Protein content can also decrease during the microwave exposure due to the fact that protein can be involved in the Maillard reaction together with the lactose. Among the proteins, the lysine is the most implicated in this reaction and its diminution in time became a nutritional indicator for the appreciation of the heat treatments on food.⁵On the contrary of heating milk in the high temperature, tends more serious loss of nutrients, major vitamins, vitamin C; one can see that averages of protein did not decreased during the microwave exposure in our study. These results indicate that the total amount of protein does not diminish during microwave heating. But this study could not show the breakdown in the protein structure. The Pierce BCS protein assay combines the well-known reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium (Biuret reaction) with colorimetric specification of the cuprous cation (Cu^{+1}) using a unique reactive containing bicinchoninic acid. I did not observe a decreasing process in this investigation because total amount of nitrogen does not change even the protein structure, amino acid chain changes, in milk. At the end of the investigation, a little increasing is observed which can be explained by evaporation and increasing in density where the milk is under microwave exposure, as it is shown in Damuta's experiment.

To use microwave for heating food results from the conservation of microwave energy into heat by friction of dipole molecules (water) vibrating due to rapid fluctuation in the electromagnetic field. We did not measure the density of microwaved milk in our study. But this effect of microwave can explain our studies results. As it is shown in the investigation, amount of matter and their concentrations will increase when volume of water decreases.

The study, which measured small changes in density and totally protein amount between the microwaved and non-microwaved milk, should provide new insights on the effects of microwaved of milk proteins. Beside even if total protein amount don't change, studies, which investigate usefulness of microwaved milk proteins, may carry

us to expositional results. In milks, which are microwaved under different wavelengths, amount of whey protein can be measure.

We clearly demonstrated that the heating milk in microwave at different power is not decreasing its protein amount. Structure of protein may be change but totally amount is constant. Because of the evaporation of water in milk, totally amount of protein may be increased our study.

A study, which investigates total protein of microwaved milk and its density together, gives explanatory results about increase in protein amounts in this experiment.

IX. APPENDICES

Appendix 1:

The Pierce BCA protein Assay is a detergent – compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein. This method combines the well-known reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium with the highly sensitive and selective colorimetric detection of cuprous cation (Cu^{+1}) using a unique reagent containing bicinchoninic acid.¹⁹

BCA Assay: The BCA assay is based on protein-copper interactions, in this case to reduce alkaline Cu^{+2} to Cu^{+1} . The cinchoninic acid reagent then complexes with the cuprous ions in a 2:1 ratio to form an intensely purple compound, which is quantified spectrophotometrically at 562 nm.

¹⁹ Smith, P.K., et al. (1985). Measurement of protein using bicinchoninic acid. Anal. Biochem. 150:76-85.

Appendix 2:

Materials, which are used for preparing the two reactive solutions, are shown in the table below.

To prepare reactive A	To prepare reactive B
10 g BCA,	2g CuSO ₄
20 g Na ₂ CO ₃ H ₂ O	
1.6 g Na ₂ C ₄ H ₄ O ₆ . H ₂ O	
4g NaOH	
9.5g NaHCO ₃	
Mixing the materials with 1 liter distilled water completes process.	Mixing 2g CuSO ₄ with 50 ml of distilled water completes process.

Table that shows the equipment which is used for preparing reactive A and reactive B in this investigation.