The Effect of Using Different Vessels in Microwave on the Alteration of Total Protein Level of Milk: An FTIR Study

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Abstract

In developing world, since people have less time to cook food, microwave ovens are commonly used. Despite the fact that it's a pratic method, its advantages and disadvantes are being debated. While there have been several studies on how food is affected by heat treatment or effects of microwave on nutritional values of food, there is less knowledge as to how does using different vessels in microwave affect the nutritional values of food. Thus, this paper aimed to observe the effect of using different vessels in microwave on food and used milk as a vehicle for this purpose. Since "food" and "nutritional value" are broad concepts, research was narrowed down to "milk" and "total protein level alteration". Since very little research has been done on how protein value of pasteurized milk may be affected, the scope of the experiment was limited to brand Sütaş®'s pasteurized milk and 5 vessels that can be used in microwave: glass, porcelain, plastic, Styrofoam and carton. Milk was microwaved in these 5 vessels respectively and milk samples were scanned using an FTIR Spectrometer. FTIR Spectrometer was connected to Perkin Elmer Spectrum® programme on the computer. As a result of the scans, the programme drew and absorbance versus wavelength graphs. Results were analyzed by interpreting these graphs.

In absorbance versus wavelength graphs, areas under amide peaks are related with total protein level. Thus, changes in these area values are associated with total protein level alteration. As a result of this experiment, it was found that the maximum total protein level alteration was in milk that was heated in plastic whereas the minimum alteration was in milk that was heated in glass. Based on the results, it was evaluated that as vessels reflect microwaves, they transmit more heat to the organic matter that is being heated in them. Since glass is thicker than plastic, it absorbs more microwaves and transmits less heat to milk and eventually causes less alteration in the total protein value of the milk. Overall, all results supported the hypothesis that as the absorbance of vessels increase, alteration in the total protein level decreases.

350 words

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Introduction

The first time I was introduced with the topic of this extended essay was while I was reading newspaper. On the health section, it was written that we shouldn't prefer microwave for heating our food since it reduces nutritional value of it and if we do, we should prefer glasses rather than plastics to heat our food in. As a person who constantly uses microwave and interested in this topic, I wondered, why should we prefer glasses rather than plastics? I began my research by searching the materials that can be used in microwave. I found that glass, porcelain, plastic, Styrofoam and carton can be used in it. As I continued to my research, I was surprised to find that water, fats and sugars absorb microwaves whereas plastics, glass or ceramics do not. I also found out that metals reflect microwaves, which is why they cause spark in microwave ovens[1] and aren't appropriate for using in them. Which made me think, why and how does these different materials affect our food?

We use different vessels in microwaves to heat our food in and simply our food consists of carbohydrates, proteins and fat. Proteins are one of the most important groups of molecules for our body. They are complex chains of smaller molecules called amino acids. Proteins are associated with motion, the basic quality of animal life. What sugars and carbohydrates are to plants, proteins are to animals.[2] Since proteins are this much important to animals, I wanted to include proteins to my research and when I think of proteins, milk is the first nutrient that comes to my mind. Milk is a white liquid produced by the mammary glands of mammals. It is the primary source of nutrition for young mammals before they are able to digest other types of food. Early-lactation milk contains colostrum, which carries the mother's antibodies to the baby and can reduce the risk of many diseases in the baby. It also contains many other nutrients [3] such as proteins. The

[1] Principles of Microwave Oven, Seong-Kyun Lee, Department of Electrical and Electronic Engineering, Yonsei University

[2] http://drlwilson.com/Articles/PROTEIN2.html, The Importance of the Protein by Lawrence Wilson, MD, May 2012, The Center For Development

[3] Pehrsson et al (2000). "USDA's National Food and Nutrient Analysis Program: Food Sampling". *Journal of Food Composition and Analysis, p:* 379–389.

total protein component of milk is composed of numerous specific proteins. The primary groups of milk proteins are the caseins. All other proteins found in milk are grouped together under the name of whey proteins. The major whey proteins in cow milk are beta-lactoglobulin and alpha-lactalbumin.[4]

The reason I started to my research was that I wondered the effect different materials to nutritional value of food in microwave. Since "food" is a broad term, because of the reasons listed above, I narrowed it down to "protein". Since I chose to observe the change in the protein levels, I determined to use milk since it includes high amount of proteins and it is a substance easy to find and work with. After learning about the protein content of milk, I realized that I don't have much knowledge about caseins or whey proteins. And since I didn't have enough information about specific proteins in milk, I decided to work with the alteration of the total protein value of it. Thus, I determined to observe how does different vessels affect milk's total protein level alteration after being treated with heat in microwave. However, it is important to note that observing total protein value of milk was not my principal aim. My main aim was to observe the effect of using different vessels in microwave and milk protein was going to serve to show this effect. After doing research, I realized that while there have been several studies on how milk is affected by heat treatment or effects of microwave on nutritional values of food, there is less knowledge as to how does the total protein content of milk changes after being heated in different vessels. This is also one of the reasons why I decided to perform an experiment about it.

All in all, at the beginning I thought why we should prefer glasses in microwave as it was mentioned in a newspaper article. Then, I did research about the vessels that can be used in microwave, general content of our food, why are proteins important for our body and characteristics of milk. Since I wondered the effect of using different vessels on nutritional value of

[4] http://classes.ansci.illinois.edu/ansc438/milkcompsynth/milkcomp_protein.html

food at first, I then narrowed my research topic from “food” to “milk” and from “nutritional value” to “total protein content”. Consequently, this paper will focus on the research question: ***How does using glass, porcelain, plastic, Styrofoam and carton vessels in microwave affect the alteration of the total protein content of pasteurized milk that is analyzed by FTIR?*** and will discuss how the experiment was designed and performed, as well as evaluating the results and will also attempt to analyze their possible consequences.

Hypothesis

For microwave approval, the ratio of plastic surface area to food, how long the container is likely to be in the microwave, how often a person is likely to eat from the container, and how hot the food can be expected to get during microwaving are estimated. The scientists then measure the chemicals that leach out and the extent to which they migrate to different kinds of foods. Only containers that pass this test can display a microwave-safe icon. Glass and ceramic are microwave-safe. However when food is wrapped in plastic or placed in a plastic container and microwaved, substances used in manufacturing the plastic may leak into the food.[5]

As the microwave absorbance of vessels increase, they absorb more microwaves and transmit less heat to food, however as the absorbance of vessels decrease, they transmit more microwaves and transfer more heat to the organic matter in them. Since glass is thicker than plastic, it is expected that it will absorb more microwaves, and transmit less heat to milk in it. Eventually, since the temperature of milk in it would not be as high as the milk in plastic vessel, the alteration of total protein value for glass vessel would be less. Additionally, for the reason that glass and porcelain are labeled as “microwave-safe” whereas plastic is not, it can therefore be hypothesised that the most alteration in total protein level will occur in the milk that is heated in a plastic vessel whereas the least alteration will be in glass or porcelein.

[5] http://www.health.harvard.edu/fhg/updates/update0706a.shtml

Method Development and Planning

A suitable method should be designed for the experiment to be performed. However, designing an appropriate method in order to answer the research question and support or reject the hypothesis brought various problems with it.

First of all, there were plenty of protein analysis techniques. First method was Kjeldahl Method. In this method, food is titrated with a strong acid and nitrogen is released. Then, the protein amount is calculated from this nitrogen content by using a conversion factor (F), however, since each protein has a different conversion factor, this factor is only an estimate. Also, it does not give a measure of the true protein, since all nitrogen in foods is not in the form of protein.[6] Secondly, I considered using one of the UV-Visible Spectroscopy techniques. However, these methods modify protein structures physically[7] and I thought this might alter the results since I was going to compare the amount of change in the protein levels and I didn't want the method itself to do any additional alteration. Last but not the least, I have gained information about Infrared Techniques. They make fast analyses, don't give any damage to the sample, preparing samples for them isn't time consuming, and small amounts of samples were enough for them. Another problem about Infrared Spectroscopy was to find its machine. After contacting some laboratories in Ankara, I found out that METU Biology Department Laboratories had *FTIR: Fourirer Transform Infrared Spectroscopy*. All in all, regarding to Infrared Spectroscopy's advantages, I decided to use FTIR in my experiment.

6 and 7 http://people.umass.edu/~mcclemen/581Proteins.html

In FTIR spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is transmitted. The resulting spectrum represents a fingerprint of a sample with absorption peaks, which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. Because each different material is a unique combination of atoms, no two compounds produce the exact same infrared spectrum. Therefore, infrared spectroscopy can result in a positive identification of every different kind of material. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present.[8]

After choosing FTIR and learning its working principle, an additional problem has occurred. FTIR Spectrometer draws graphs based on the bonds between molecules. However, proteins aren't the only structures in milk. Milk also contains high amount of water. These water molecules' bonds would also appear in the spectrum. Since I wanted to measure the alteration of total protein level, I didn't want water molecules' spectra to interfere protein spectrum. Then, I came up with an idea: I would centrifuge a milk sample until a precipitate occurs, and analyze the upper-watery part of this sample, and would see if this part contains any proteins or not. And if they don't contain proteins, then I would use these samples as buffers and by substracting these buffers from each spectrum, I would obtain milk spectra without any water bonds.

Following the problem about water molecules bonds, another minor difficulty was about choosing the vessels that the milk samples will be heated in. Glass, porcelain, carton, Styrofoam and plastic vessels were chosen since they were allowed for microwave-use. Metal or aliminum vessels weren't chosen since they reflect microwaves and might cause sparks in microwave ovens.

[8] www.thermonicolet.com

Now it became important to make sure that all variables were being controlled. Type of milk, amount of milk, power of microwave, time of heating, used plates in FTIR and humidity of the room were the most apparent of these variables and were dealt with accordingly. First of all, type of milk was controlled; a full fat, pasteurized cow-milk of the brand *Sütaş* was used. It was important to use the same type of milk in each trial since different brands' different types of milks' ingredients (e.g. fat level, protein level) differ. Secondly, the amount of milk that was heated in microwave was controlled. In each vessel, 200 mL milk was heated. Thirdly, while preparing samples for FTIR, same amount of milk was used. Since milk samples were sandwiched between two plates, 30 µL overbrimmed and there were too many spaces left when 10 µL was used. Thus, it was decided to use 20 µL milk in each trial. Different amounts of milk would have contained different amounts of organic bonds leading to different total-protein levels in the spectra. Another variable was about the heat treatment. To ensure that each sample was treated with the same power of heat for the same amount of time, milk was heated the same amount of watts for equal time lengths. If this wasn't controlled, I wouldn't have been able to compare same heat treatment's affect on milk in different vessels. However, until I have performed my 1st trial, I couldn't decide on values. Moreover, to avoid any additional molecules' bonds on the specta, I had to control two things; the plates used in FTIR and the humidity of the room. Since FTIR analyzes samples based on the molecular bonds in them, it was important to make sure that milk protein was the only sample that was producing spectra. So, I used CaF_2 plates, which were transparent to Infrared light and did not introduce any additional lines on the spectra. The last variable I controlled was the room's humidity and its reason was similar with the reason why I used CaF_2 plates; in order not to introduce any other lines to the absorbance graphs. To adjust room's humidity, I made sure that the special chamber's (which FTIR was placed in) door was always closed and I did background scans to substract the bonds of the molecules in the air from the spectra. Finally, it was intended to keep vessel sizes constant, however since they were ready-made, it was impossible to keep their sizes

constant. Despite the fact that microwave doesn't heat only food surface but heats food evenly due to its working principle, it was paid attention to choose at least similar sized vessels.

Despite the fact that I determined how to control the controlled variables before I performed my experiment, I couldn't decide the power of microwave and the duration of heating until I have performed the first few trials. Since people use microwave to heat milk for drinking, I tried to find a power value that heats the milk to the generally preferred drinking temperature. After performing some trials, I decided that 180 watts was a suitable power value. However, duration of heating was still a problem. First, I tried to heat 5 minutes in 180 watts: milk was too hot that it bursted to the walls of microwave. Then I tried 30 seconds: however this time milk was too cold that it could hardly be called as "heated". Then I tried 2 minutes: the result was like I wanted. Also, it was a short time value that was efficient while doing large numbers of trials. Until heated milk samples were used in trials, they were kept in room temperature.

To minimize experimental errors, 5 trials were made for each vessel. In addition, in order to make sure that there weren't any milk remainders on the plates from previous trials, plates were hygienized after each trial.

Method

Materials and Apparatus

- ✓ FTIR Spectrometer
- ✓ 4 CaF_2 plates
- ✓ Stabilizer plastic for FTIR
- ✓ Micropipette (adjusted to 20 µL)
- ✓ 25 micropipette tips
- ✓ *Sütaş* full-fat milk (1 L)
- ✓ Microwave
- ✓ Graduated cylinder (300 mL)
- ✓ Glass vessel
- ✓ Porcelain vessel
- ✓ Plastic vessel
- ✓ Styrofoam vessel
- ✓ Carton vessel
- ✓ Distilled water
- ✓ Detergent
- ✓ Kettle
- ✓ Tap water
- ✓ Napkin

Since CaF_2 plates are gentle, they require warm water while sterilizing them. Before each trial, sink was filled with half tap water half boiled water from kettle. Detergent was added to sink. CaF_2 plates were washed first in this warm detergent-water, and then rinsed in tap water, lastly rinsed with distilled water again. To dry the plates, a thin-napkin was used and double-checked in order not to make a remainder of detergent or dust particle on it.

After sterilizing CaF_2 plates, 200 mL milk was heated in glass vessel in microwave at 180 watts for 2 minutes. The stabilizer was placed on a plate. Using a micropipette that was adjusted to 20 μL, milk was pourred on the plate, a second plate was placed over it and milk was sandwiched between two CaF_2 plates.

This sandwiched milk sample was placed in FTIR. FTIR completes scans in approximately 5 minutes. In order to use time efficient, during this time, a second sample was prepared. When the scan was completed, this sample placed in FTIR and during this scan, the used plates were sterilized as explained above and another sample was prepared.

The whole procedure was performed for porcelain, plastic, Styrofoam and carton vessels and was repeated 5 times for each vessel in order to minimize any error made.

Figure 1: Process of preparing samples for FTIR

Data Analysis

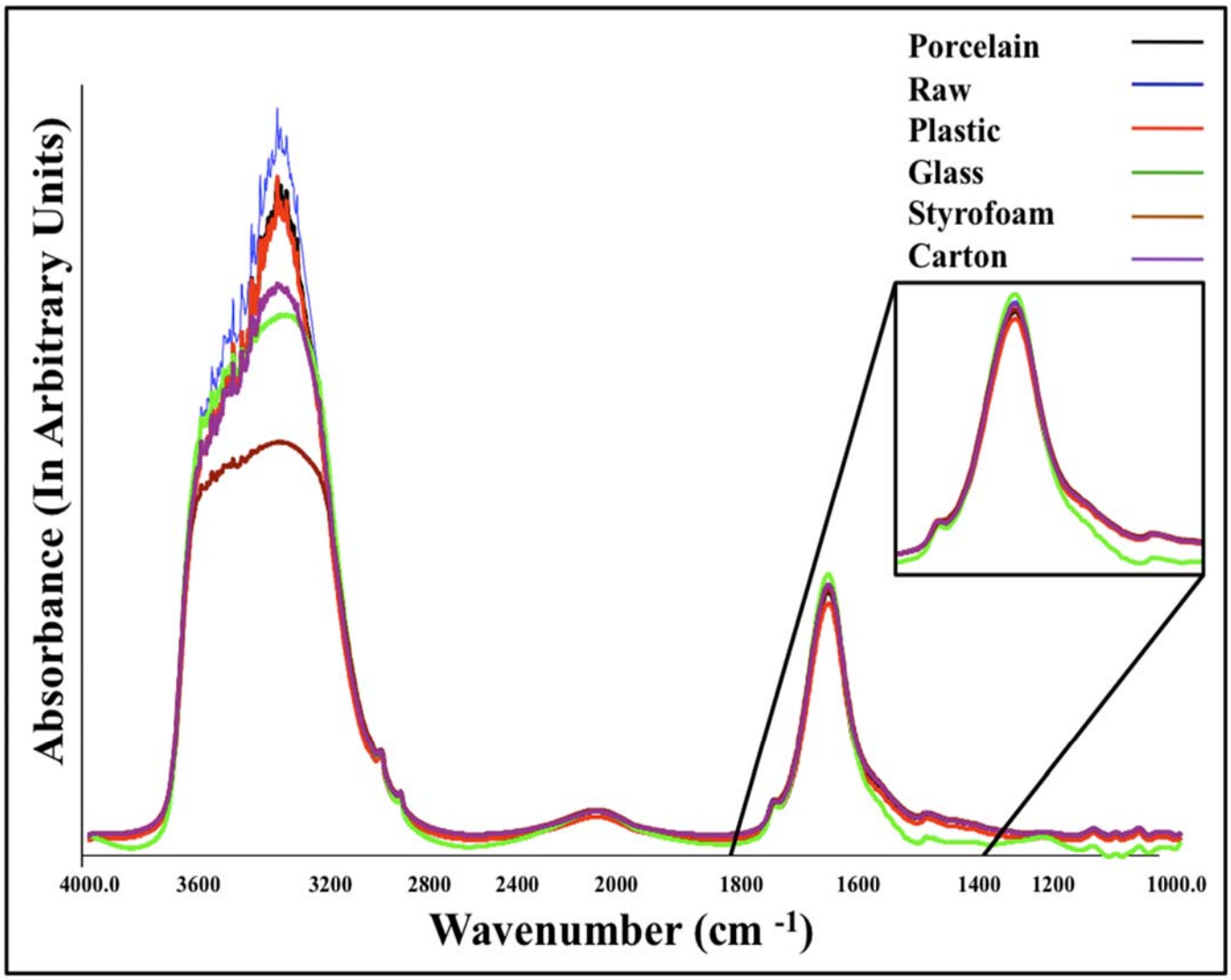

Figure 2: Absorbance versus wavenumber graph drawn by PerkinElmer Spectrum® programme which is connected to PerkinElmer® FTIR Spectrometer. Note that the peak between 1800 cm^{-1}-1400 cm^{-1} wavenumbers is amide band. Graph is labelled in Microsoft Powerpoint programme.

Areas under amide II band were calculated by using PerkinElmer Spectrum® and are presented below.

Type of Vessel	Amide II interval (cm^{-1}) (± 0.01)	Trials	Area under amide II peak (in arbitrary units) ± SEM
Glass	1481.75 – 1427.57	1	19.110 ± 0.906
		2	18.990 ± 2.775
		3	19.160 ± 2.656
		4	16.190 ± 2.763
		5	20.430 ± 2.954
Plastic	1473.71 – 1437.16	1	15.410 ± 1.038
		2	12.450 ± 3.176
		3	11.870 ± 2.922
		4	12.560 ± 3.339
		5	11.530 ± 3.325
Porcelain	1481.81 – 1433.45	1	15.000 ± 1.228
		2	14.760 ± 0.504
		3	15.000 ± 0.973
		4	14.350 ± 1.088
		5	17.330 ± 1.036
Styrofoam	1477.96 – 1432.99	1	15.760 ±1.281
		2	20.770 ± 0.206
		3	13.640 ± 1.364
		4	16.170 ± 1.061
		5	17.430 ± 1.323
Carton	1482.00 – 1436.79	1	19.400 ± 0.190
		2	17.040 ± 0.350
		3	13.350 ± 2.115
		4	19.600 ± 1.430
		5	14.670 ± 2.275
Raw Milk (without heat treatment)	1481.71 – 1431.28	1	19.780 ± 0.906
		2	36.300 ± 2.775
		3	17.580 ± 2.656
		4	16.740 ± 2.763
		5	19.220 ± 2.954

Table 1: Table showing the areas under amide II peaks in graphs that were drawn by PerkinElmer Spectrum®. Amide II region is different for each group since starts and ends were individually decided for each group. Error values were shown as “SEM” : “Standart Error of Mean” and SEM was calculated by using GraphPad Prism 5® programme.

Statistical Analysis

One-way analysis of variance	
P value	< 0.0001
P value summary	***
Are means signif. different? (P < 0.05)	Yes
Number of groups	6
F	16.06
R squared	0.7698

Table 2: One-way analysis of variance table drawn by GraphPad Prism 5.

ANOVA Table	Sum of squares	Degrees of freedom	Mean square
Treatment (between columns)	784.5	5	156.9
Residual (within columns)	234.5	24	9.772
Total	1019	29	

Table 3: ANOVA table drawn by GraphPad Prism 5.

Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary
glass vs plastic	15.19	10.87	Yes	***
glass vs porcelain	3.792	2.712	No	ns
glass vs styrofoam	2.8	2.003	No	ns
glass vs carton	1.95	1.395	No	ns
glass vs raw milk	0.83	0.5937	No	ns
plastic vs porcelain	-11.4	8.153	Yes	***
plastic vs styrofoam	-12.39	8.862	Yes	***
plastic vs carton	-13.24	9.47	Yes	***
plastic vs raw milk	-14.36	10.27	Yes	***
porcelain vs styrofoam	-0.992	0.7096	No	ns
porcelain vs carton	-1.842	1.318	No	ns
porcelain vs raw milk	-2.962	2.119	No	ns
styrofoam vs carton	-0.85	0.608	No	ns
styrofoam vs raw milk	-1.97	1.409	No	ns
carton vs raw milk	-1.12	0.8011	No	ns

Table 4: Tukey's Multiple Comparison Test results table drawn by GraphPad Prism 5.

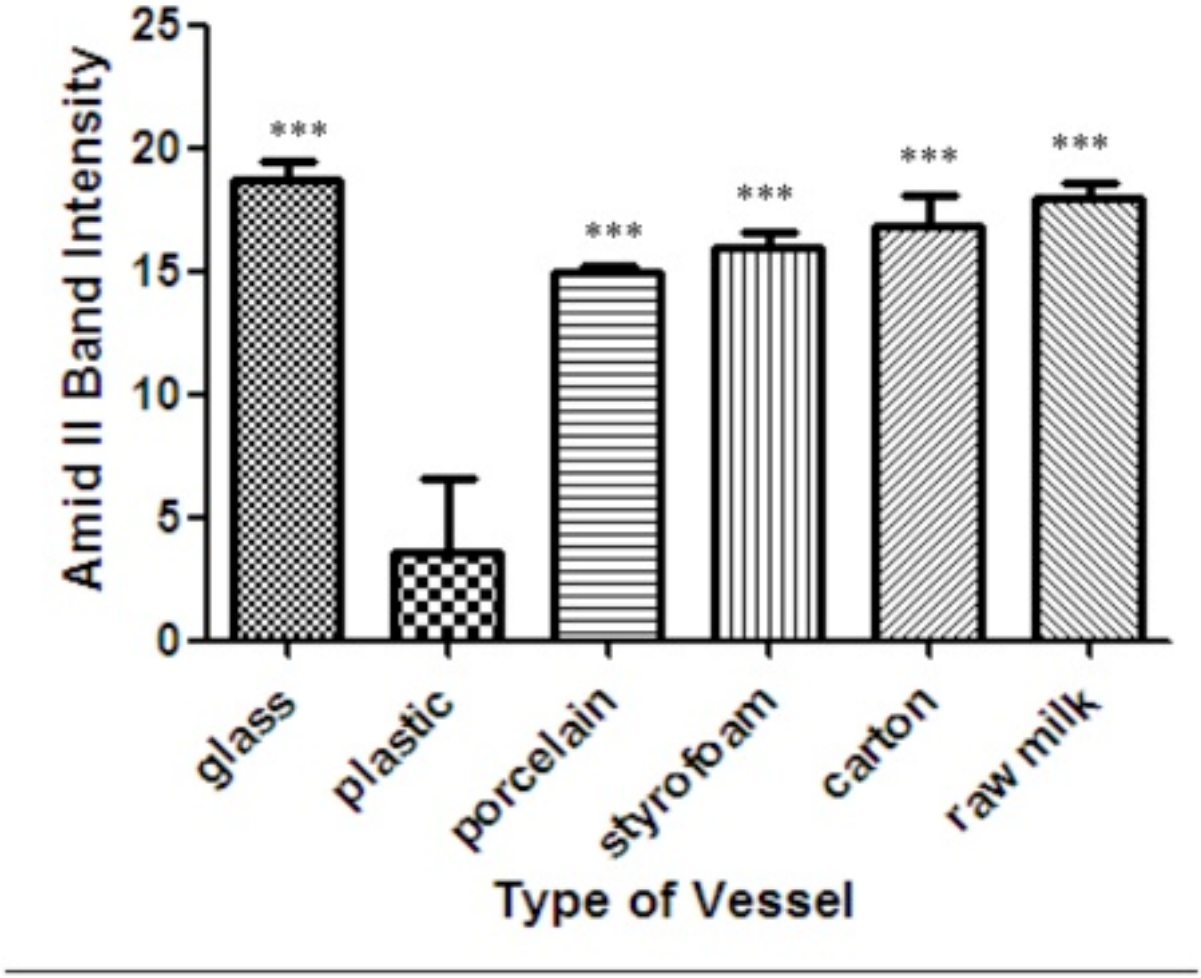

Figure 3: Graph showing the Amide II band intensity of milk which was treated in glass, plastic, porcelain, styrofoam, carton and raw milk in microwave. Note that *** shows the groups that have significant protein level alteration compared to plastic. Graph is drawn by GraphPad Prism 5 ® as a result of the statistics calculations in Table 2.

Evaluation

This paper focused on the research question: *How does using glass, porcelain, plastic, Styrofoam and carton vessels in microwave affect the alteration of the total protein content of pasteurized milk that is analyzed by FTIR?* Results support the hypothesis that the most alteration in total protein level of milk is when it is heated in a plastic vessel in microwave and the least alteration is in glass vessel. As a matter of fact, they conform to the suggestion (see Hypothesis) that as the absorbance of vessels increase, the alteration in protein level decreases. As vessels reflect microwaves, they transmit more heat to milk that is being heated in them. Since glass is thicker than plastic, it absorbs more microwaves and transmits less heat to milk in it and minimizes total protein level alteration.

In order to analyze results, some relevant information should be given about amide bands. The amide I band which is located at 1642 cm^{-1} wavenumber in fingerprint region, originates from proteins consist of C=O, C-N and N-H bonds' vibrations. The amide II band which is located at 1551 cm^{-1} wavenumber, also derives from proteins with N-H and C-N vibrations. Changes in amide I and amide II bands give information about alterations in protein secondary structures.[9] Changes in the areas of amide bands indicates total protein level alteration. For instance it can be seen in figure 2 that area under glass' amide curve is the largest whereas area under plastic's amide curve is the smallest. It can also be seen in figure 3, which was drawn by the area values of curves that plastic's amide II band intensity is the smallest while glass' is the largest. These protein alterations are related with alterations in protein structure. Since results show that there were alterations in amide II band concentrations, one might say that N-H bending and C-N streching structures (secondary structures) may have denaturated and denaturation might be the reason of total protein level alteration.

[9] Şen, İlke. *Macromolecular Characterization of Adipose Tissues in Inbred Obese Mouse Models.* Master of Science Thesis. The Graduate School of Natural and Applied Sciences of Middle East Technical University, 2012.

In statistical analysis, the differences in variance were analyzed using one-way ANOVA test. Tukey's Multiple Comparison test was used as a post-hoc test and the results of each group were compared with each other. The **p values** less than or equal to 0.05 were considered as statistically significant. $\mathbf{R^2}$ value is the fraction of overall varience and it was calculated as **0.7698** (see Table 2). This small R^2 value indicates a small fraction of variation. In ANOVA table (see Table 3), by dividing sum of squares values to degrees of freedom, mean square values were calculated. **F ratio** is the ratio of two mean square values and it was calculated as **16.06**. This large F ratio value suggests that there are significant differences between treatments. Also, Tukey's Multiple Comparison Test (see Table 4) that was performed indicated that alterations in protein level in different vessels are only significant when compared to plastic group. Thus, it should be noted that there is no significant difference between any other two groups.

It should be mentioned that, in order to obtain significant results, a slight change was made on Method Development and Planning part. It was mentioned that in order to subtract water bonds' spectra from milk spectra, buffers were going to be prepared and subtracted from each milk spectra. This procedure was performed, however there were no significant result obtained in any group according to Tukey's Multiple Comparison Test. Thus, it was decided not to subtact anything from any spectrum. Because, in the end, alteration in protein levels was going to be compared to each other. Despite the fact that milk spectra included water bonds' too, since every milk spectra contained them and they were going to be compared to each other, the decision of not using a buffer spectra was made. However, if this experiment was to be repeated, ammonium sulfate precipitation method could have been applied before the centrifuge. Adding ammonium sulfate to a protein containing solution neutralizes surface charges. Charge neutralization means that proteins will tend to bind together, form large complexes and hence are easy to precipitate out by mild centrifugation. Since each protein will start to aggregate at a characteristic salt

concentration, this approach provides a simple way of enriching for particular proteins in a mixture.[10]

Despite the fact that results proved the hypothesis, there were some sources error. One of the most important effects of these errors can be seen in figure 3. Even though raw milk and glass groups are not significant when compared to each other but only significant when compared to plastic, raw milk's protein intensity is lower than glass group's protein intensity. Since a milk sample that wasn't treated with heat cannot have less protein amount than a milk sample that was treated with heat, there is an important error. It could be a result of error in micropipette's volume. There may have been less amount of milk samples in raw milk trials than glass group's trials due to errors in volume calibration of pipette. Also, it might be related with hygenization. Regardless of the hygenization process, there might have been some milk residues from previous trials on CaF_2 plates. Experiment also includes another error sources; to begin with, experiment was performed in 2 different days and 2 different boxes of Sütaş® milk was used. Different boxes of the same brand may include some ingredient differences and this might have affected the protein level calculations. Secondly, volume of vessels wasn't kept equal since microwave doesn't heat only the top of the food but heats all surfaces of food equally. However, vessels of different sizes might have influenced the amount of protein denaturation and introduced some errors to calculations. Additionally, FTIR Spectrometer is affected by changes in room temperature. Since temperature changes humidity level, number of air molecules' bonds also changes. To minimize this effect, background scans were performed before starting the experiment. However, these scans weren't repeated before each trial. Changes in the temperature of the room, after background scan, might have had an effect on milk spectra. Last but not the least, not being able to use same sized vessels might have affected the results. Since ready-made vessels were used, despite the fact

[10] http://www.encorbio.com/protocols/AM-SO4.html

that their sizes were similar, sizes were not equal and this might have resulted in some additional errors.

If this experiment was going to be repeated, in order to minimize experimental errors, some improvements may be applied. First of all, to minimize pipette's volume errors, a digital micropipette that has an absolute volume calibration may be used. Secondly, to reduce the chance of having different samples of milk, experiment may be completed in 1 day and the same box of milk may be used in each trial. Thirdly, in order to prevent any milk residues on plates, hygienization process might have included an increased amount of detergent and increased time of rinsing. Also, to make sure that size of vessels do not introduce any errors, same sized vessels might be used. Also, to unable air molecules' spectra to interfere milk spectra, more background scans could be performed. Lastly, in order to prevent any error due to different sizes of vessels, equal sizes of different vessels might be preferred. Nevertheless, it should be mentioned that the method itself (FTIR Spectroscopy) was the most suitable method for this experiment and was preferred to any other method as a result of its considerable advantages that were discussed in Method Development section. Thus, if this experiment was to be repeated, the method wouldn't be changed.

Conclusion

This experiment answered the research question and proved the hypothesis. However, there are some questions that have to be asked after obtaining results that suggest that the protein value of milk that was heated in microwave is affected by the type of vessel that it was heated in. For instance, does using different vessels have an affect on also the carbohydrate, fat or vitamin values of food? Russian researchers reported a marked acceleration of structural degradation leading to a decreased food value of 60 to 90% in all foods tested. Among the changes observed were: Decreased bioavailability of vitamin B complex, vitamin C, vitamin E, essential minerals and lipotropics factors in all food tested.[11]

It would be interesting to investigate whether using different vessels in microwave have an affect on the nutritional value of breast milk. Young Families, the Minnesota Extension Service of the University of Minnesota, attempt to answer this in their article: Heating the bottle in a microwave can cause slight changes in the milk. In infant formulas, there may be a loss of some vitamins. In expressed breast milk, some protective properties may be destroyed.[12]

Today, there is a debate concerning the usage of conventional and microwave ovens. What would happen if a conventional oven were used in this experiment instead of a microwave oven? In 1991, Dr. Hans Ulrich Hertel et al. published a research paper indicating that food cooked in microwave ovens could pose a greater risk to health than food cooked by conventional means. [13]

[11, 11 and 12] http://curezone.com/foods/microwave_oven_risk.asp

As yet no-one really seems to be able to suggest all the outcomes of using different vessels in microwave ovens, it will mean doing a lot more research to find out the exact extent of the harm microwaves are doing to nutritional values of food and maybe to human health.

Appendix

Figure 4: Photo of the milk *(Sütaş)* and vessels (from left to right): glass, carton, porcelain, Styrofoam and plastic

Figure 5: Micropippete, CaF_2 plates, stabilizer and milk in the glass vessel

Figure 6: Fourier Transform Infrared (FTIR) Spectrometer