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THESIS OF PhD DISSERTATION

Summary

Treatments of liquid foodstuffs with special regard to the effect of microwave on milk

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CONTENTS

1. RESEARCH AIMS

The dissertation reports our microwave research activities on foodstuffs. First aim was to establish the analytical application of microwave treatments. In order to accomplish this, homogeneous microwave field had to be elaborated inside the treated materials. In this case, the development of a new milk fat content determination method was enabled. This method combines different physical treatments, and fulfills the reliable accuracy of existing reference method requirements. Based on the results it was presumed that not only the heating effect of microwave, but the so called “non-thermal” effects also play role in the material. These effects, their influences on the measurements and the harmfulness of these effects had to be examined.

2. MATERIALS AND METHODS

2.1. Examinations of temperature distribution

The temperature distributions inside different materials during microwave radiation were examined. At the beginning treatments were carried out at the beginning with an own-built microwave unit. Latter on, a Panasonic NNF 653WF inverter type microwave oven with a FISO MWS-4 optical thermometer was used for the measurements. Infrared images were taken from the treated materials, in order to follow the temperature distribution.

2.2. Development of homogeneous microwave field

Due to the uneven warming up (irregular temperature distribution) of the samples placed inside the microwave oven water traps were used to homogenize the microwave field. The container with the sample material was placed in the middle of the turntable. The surrounding four water containers were placed around the sample holder evenly, so that they created homogeneous field distribution. Several combinations of different geometric sample holders with different liquids were tested in order to find the best positions of the four water traps for the most even warm up of the sample materials.

2.3. Determination of fat content of skimmed and raw milk samples

The fat contents of raw and skimmed milk samples were determined by using a combined (microwave and convective) treatment. The microwave treatments were on 100 W for 25 minutes. After this, the samples were placed into an experimental drying tunnel. The skimmed milk samples were dehydrated on 30 °C and 2 m/s for 90 min., while raw milk samples were treated on 40 °C and 1.5 m/s for 120 min. During drying the control and data collection were carried out by LabView software. The evaluation of raw date was done by Matlab 7.0, in more specific we determined the dehydration curves and linear fitting of a special section of the curves.

2.4. Microscopic examinations

Skin is forming on the top of the milk in the drying tunnel. The thickness and area of this milk skin depends on the fat content of the milk. It was presumed that the structure of milk skin and the constituents of milk (first of all fat content) are

changing after heating. Microscopic examinations were arranged to reveal these changes. Scanning electron microscope (Philips XL30 ESEM) was used to detect the structure of milk skin heated up on hot plate or microwave. Whereas, optical microscope was used to follow the diameter changes of fat globules.

2.5. Examinations of lipase and xanthine oxidase enzymes in milk

Significant changes in milk fat globule diameter happened 30 minutes after the microwave radiation. This led to the conclusion that enzymatic activities have to play role in fat globule diameter changes. Lipase and xanthine oxidase (XO) enzymes activity changes were measured in conventionally and microwave heated, non-heated (control) of skimmed milk (3.6% fat content) by using HPLC and spectrophotometer. Pure enzyme suspension was also measured (conventionally and microwave heated, control) in order to detect the pure effects of treatments on enzymes by eliminating the disturbing factors of other milk constituents.

3. RESULTS

3.1. Examination of temperature distribution

Major temperature differences inside materials were detected during microwave treatments. In case of distilled water this difference could be 5.2 °C, while in denser materials (e.g. ketchup) this could reach even 56 °C (Fig. 1).

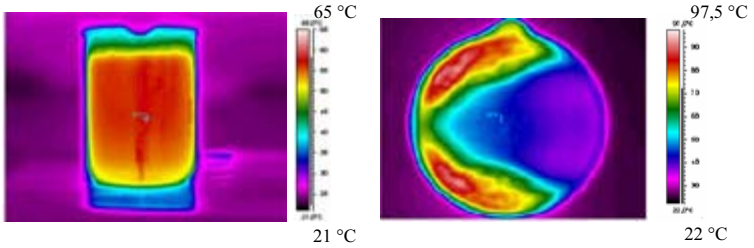

Figure 1 Infrared images of microwave heated distilled water (left) and ketchup (right).

The shape of sample holders could also highly affect the temperature distribution besides the physico-chemical parameters of the material.

3.2. Development of homogeneous microwave field

Homogenous microwave field had to be developed around the sample holder inside the microwave oven. This was accomplished by using water containers as water trap around the sample holder. The homogeneous microwave field distribution was detected by the uniform temperature rise of the sample. The position of water traps (Fig. 2) are influenced by the shape of the sample holder and the quality and quantity of the sample material. In optimal case the maximum temperature difference inside the material was only 2.6 ° after the radiation.

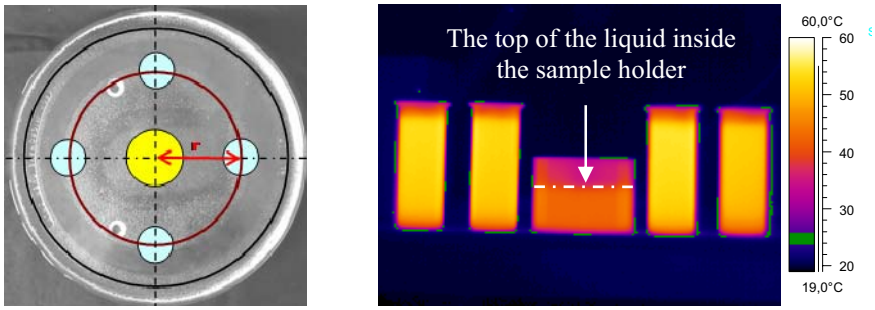

Figure 2 The position of the water trap on the turntable.

If the sample material was homogenized milk the distance of the water trap containers from the center was 10.85 cm.

3.3 Determination of fat content of skimmed and raw milk samples

The basic of our fat content determination method is the joint application of microwave and convective treatment. After microwave heating the dehydration curves of skimmed and raw milk in the convective drying tunnel can be seen on Fig. 3.

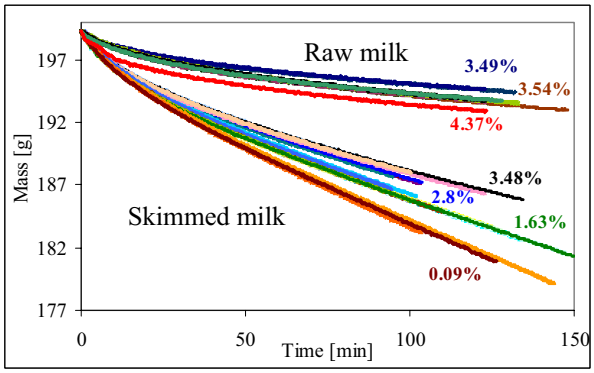

Figure 3 Dehydration curves of skimmed and raw milk samples.

The drying velocities were calculated between 40 and 90 minutes of the drying by skimmed milk, whereas by raw milk it was between 75 and 120 minutes. The calibration curves were determined by linear fitting of a given section of the dehydration curves (Figs. 4 and 5). The calibration curves represent fat content (*FC*) as a function of the slop the equations.

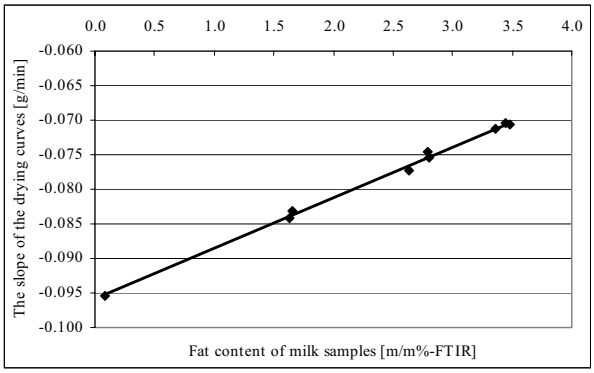

Figure 4 The slope of the section (between 40 and 90 minutes) of dehydration curves as a function of fat content of skimmed milk samples.

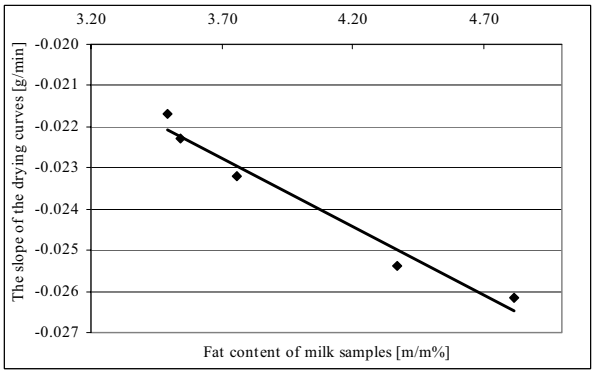

Figure 5 The slope of the section (between 75 and 120 minutes) of dehydration curves as a function of fat content of skimmed milk samples.

Rearranging the equations of the lines given on the Figures 4 and 5 gives the final calibration equations for the fat content:

By skimmed milk (R^2=0,996):

$$FC = 135{,}77 \cdot \alpha + 13{,}026 \quad (1)$$

By raw milk (R^2=0,97):

$$FC = -293{,}75 \cdot \alpha - 2{,}9796 \quad (2)$$

where:

FC: the fat content of the milk [m/m%];

α: the slope of the dehydration line.

By using the calibration equations (1) and (2) and determining the slop of dehydration curve (tgα) of given milk sample the fat content can be determined with the required accuracy. Because of the tgα values of raw and skimmed milk samples are different therefore an unknown origin milk sample can also be determined.

3.4. Microscopic examinations

The tendencies of slopes determined from dehydration lines of skimmed and raw milk samples are different as it can be seen on Figs 4 and 5. The reason of this is the structural differences of different milk.

Based on the electron microscopic examinations, it turned out that there is significant correlation between the structure of milk skin and the fat content of the milk samples. In case of both the skimmed (Fig. 6) and the raw milk (Fig. 7) the marks of air bubbles and vapor on milk skin are increasing with the increasing fat content.

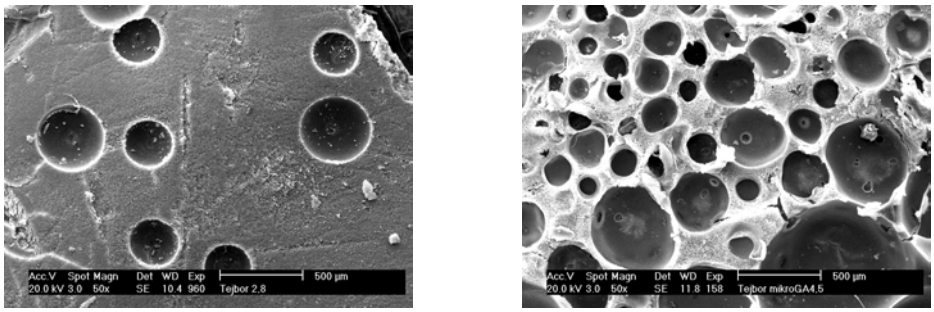

Figure 6 Scanning electron microscopic images of milk skins of a 2.8% (left) and a 3.6% (right) fat content skimmed milk samples. Magnification: 50×.

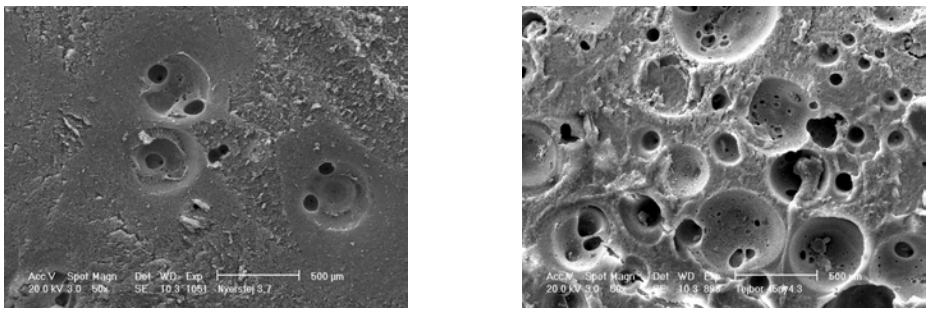

Figure 7 Scanning electron microscopic images of milk skins of a 3.7% (left) and a 4.3% (right) fat content raw milk samples. Magnification: 50×.

During heating the locally forming water vapor bubbles emerge toward the surface. Meanwhile, they associate with the fat globules. The fat globules cover the skin of the bubbles, hence stabilize them. The more and bigger globules attaching with air bubbles, the more stable bubbles are formed. Hence, there is bigger chance to reach the surface. This phenomena occurs both the raw and the skimmed milk. This explains why the intensity of dehydration in raw milk is higher if the fat content is higher. In case of skimmed milk the structure of milk skin is less dominant; the evaporation is determined mainly by the surface area (size) of the skin.

The size of air bubbles is growing and the structure of milk skin is changing due to the microwave effect. This tendency can not be seen when the samples are heated on hot plates (Fig. 8). This proves the non-thermal effect of microwave.

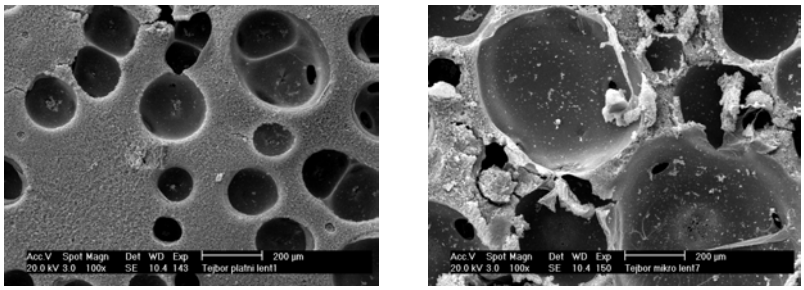

Figure 8 Scanning electron microscopic images of a 3.6% fat content milk skin heated on hot plate (left) and microwave (right). Magnification: 100×.

In the course of light microscopic examinations we experienced that the diameter of milk fat globules of skimmed milk increased but of raw milk it decreased during microwave treatment (Fig. 9).

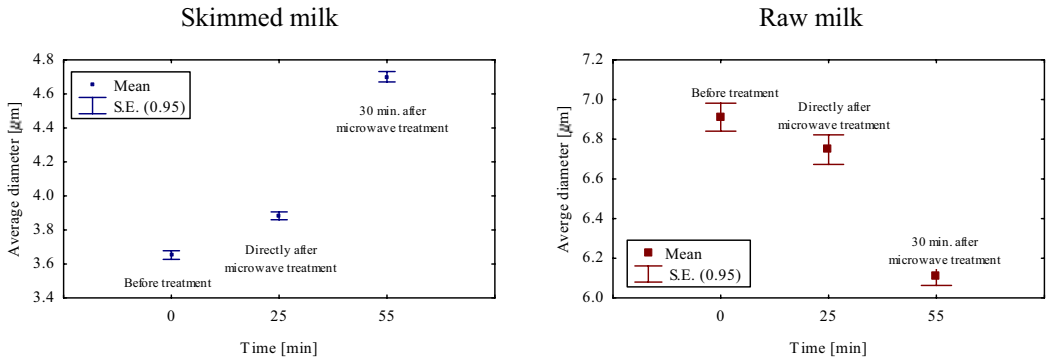

Figure 9 Changes of milk fat globule diameters before, right after and 30 minutes after microwave treatment (25 min.).

Significant milk fat globule diameter changes happened 30 minutes after the microwave treatment. We draw the conclusion from this that not only the

microwave influenced the fat globule size changes but enzymatic processes also play role.

3.5. Examinations of lipase and xanthine oxidase enzymes in milk

The first enzymes that were examined in milk were the lipases (lipoprotein lipase). High pressure liquid chromatography (HPLC) was used to detect the activity changes of free fatty acid contents in milk and in free suspension (Figs. 10 and 11).

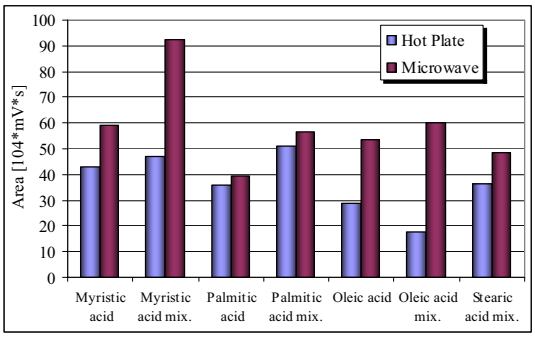

Figure 10 Quantitative changes of fatty acids (single and blends) of milk samples heated on hot plate and microwave.

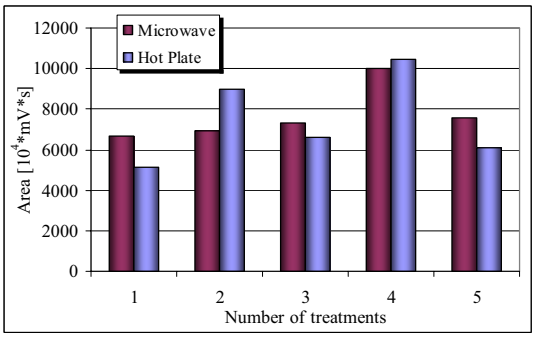

Figure 11 Quantitative changes of fatty acids of pure enzyme suspensions heated on hot plate and microwave.

There was a significant difference in changes of free fatty acids in milk but there was not found in pure enzyme suspension between the hot plate heating and the microwave heating. Based on this result, but in contrast to the literature we cannot declare that the lipase enzyme activity is increasing due to the microwave effect. Therefore, we supposed that the microwave affected another enzyme (xanthine oxidase) in connection to fat globules and so, indirectly contributed to the increase of lipase enzyme activity.

The xanthine oxidase enzyme reaction rates were measured by using the same heating parameters as the previous measurements. During the XO activity studies spectrophotometric examinations were carried out on 290 nm by detecting of the changes of hydrogen peroxide level (Fig. 12).

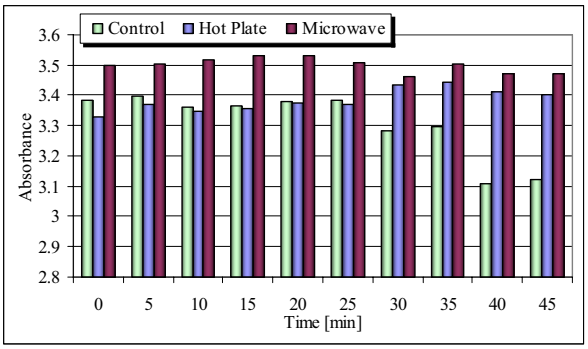

Figure 12 The changes of hydrogen peroxide contents of milk samples as a function of elapsed time after the different treatments (control, hot plate, microwave)

In case of xanthine oxidase the changes of pure enzyme suspension (heated both in hot plate and microwave, too) were also studied correlating with the control samples. In these measurements 240 nm was used to detect the changes of hydrogen peroxide (Fig. 13).

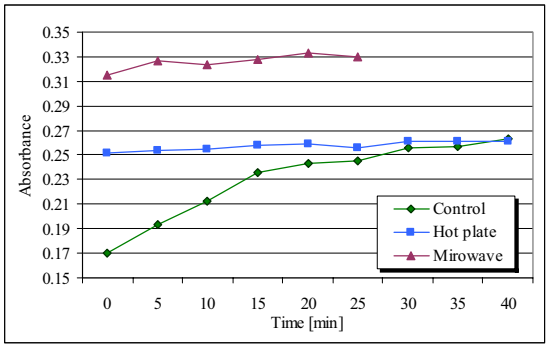

Figure 13 The average hydrogen peroxide content changes inside enzyme suspension as a function of elapsed time after the treatment.

Based on this results it can be concluded that the microwave radiation significantly increase the enzyme activity of xanthine oxidase.

By means of xanthine oxidase enzyme activity change the membrane of milk fat globules raptures, the inside triglycerides release. Outside they can contact with lipases. During heating free fatty acids (from triglycerides and lipase enzyme reaction products) can link up with the bubbles and stabilize their surfaces. As a result of this more and bigger marks of bubbles can be detected on milk skins formed on the surface. The higher milk fat content results more stable bubbles. These carry more water vapor and thus enhance the intensity of evaporation. Therefore, dehydration of the higher fat content milk samples is bigger than the smaller fat content ones. In case of skimmed milk the evaporation is mainly determined by the surface (size) of milk skin and the above mentioned phenomena is less important.

4. NEW SCIENTIFIC RESULTS (THESES)

1. Homogeneous microwave field was developed by using water trap. The maximum temperature difference inside the treated material in this field was 2.6 °C. This homogeneous field enables to use microwave for analytical purpose.

2. A method was developed to determine fat contents of skimmed and raw milk by using combined (microwave and convective) treatments. The accuracy of this method is 0.01% that is corresponds to the accuracy of the reference methods.

3. It was established that milk skin layer is formed on the surface of milk: its structure and size influence the evaporation of milk. The forming of milk skin is also determined by the heating method (conductive or microwave). Scanning electron micrographs are proving this.

4. It was proved by light microscopic studies that the average diameters of skimmed milk fat globules are enlarging, while the diameter of raw milk are decreasing. Significant differences can be proved 30 minutes after the treatment. Therefore I concluded that microwave is hardly influence directly the fat globule diameter change. The main reason behind the changes is the enzymatic activity.

5. It was proved that the xanthine oxidase enzyme activity increased in both the milk samples and pure enzyme suspensions. The lipase enzyme activity increases in milk but remains stable in pure suspension under the same

experimental circumstances. From this I proved that the microwave influences the enzymes activities in different extent.

5. LIST OF PUBLICATIONS

JOURNAL ARTICLES

1. **Lakatos, E. – Lőrincz, A. – Neményi, M (2002):** Az ultrahangos sejtroncsolás fizikai kritériumainak meghatározása a folyékony élelmiszerek csíraszámcsökkentésével kapcsolatban. Élelmezésipar. LVI. pp. 203-207.

2. **Lakatos, E. – Kovács, A.J. – Neményi, M. (2005):** Homogeneous microwave field creation. Hungarian Agricultural Engineering. No. 18. pp. 80-81.

3. **Neményi Miklós – Lakatos Erika – Kovács Attila J. (2006):** Examination of milk fat globule changes in homogeneous microwave field. Journal of Food Physics. In press.

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1. **Neményi, M. - Lőrincz, A. - Lakatos, E (2003):** Az ultrahangsugár fizikai paramétereinek változása a besugárzott anyagban. MTA-AMB 27. Kutatási-Fejlesztési Tanácskozás, Gödöllő, 2003. 01. 21-22., Proceedings, szerk. Dr. Tóth László 3. kötet pp. 66-70. (poster)

2. **Lőrincz, A. - Neményi, M. - Lakatos, E. (2003):** A magas intenzitású ultrahang sejtroncsoló hatásának alakulása a besugárzott anyagtól függő akusztikai jelenségek mellett. MTA-AMB, 27. Kutatási-Fejlesztési Tanácskozás, Gödöllő, 2003. 01. 21-22. Proceedings, szerk. Dr. Tóth László 2. kötet pp. 107-111. (poster)

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5. **Lakatos, E. – Neményi, M. – Kovács A.J. – Stépán, Zs. (2004):** Egyes folyékony élelmiszerek mikrohullámú kezelése. Műszaki kémiai Napok „04" Veszprém 2004. 04. 20-22. Proceedings, szerk. Dr. Filka Judit pp. 217-219.

6. **Neményi, M. – Lakatos, E. – Kovacs A.J. – Kacz, K. – Stépán, Zs. (2004):** Különböző zsírtartalmú tejek mikrohullámú kezelése. VI. Nemzetközi Élelmiszertudományi Konferencia, Szeged 2004. 05. 20-21. Proceedings, szerk. Hodúr Cecilia pp. 104-105.

7. **Kacz, K. – Lakatos, E. (2004):** Terménydarálók, takarmánykeverők. Agro napló VIII. évf. 8.sz. pp. 94-96.

8. **Neményi, M. – Lakatos, E. – Kovacs A.J. – Kacz, K. – Stépán, Zs. (2004):** Microwave treatments of milk with different fat content. Sustain Life Secure Survival II. Conference. Socially and Environmentally Responsible Agribusiness. Prága, September 22-25. Conference proceedings p. 76.

9. **Lakatos, E. – Kovács A.J. - Neményi, M. – Némethné Varga, M. (2004):** Mikrohullámmal kezelt folyékony élelmiszerekben a hőeloszlás vizsgálata. XXX. Óvári Tudományos Napok, 2004. október 7. Mosonmagyaróvár szerk.: Kovácsné Prof. Dr. habil. Gaál Katalin pp. 151.

10. **Lakatos, E. – Neményi, M. – Kovács A.J (2005):** Különböző anyagokról készített infrakamerás felvételek kiértékelése. MTA-AMB, 29. Kutatási-Fejlesztési Tanácskozás, Gödöllő, 2005. 01. 18-19. Proceedings, szerk. Dr. Tóth László, Dr. Vinczeffy Zsoltné (poster)

11. **Neményi, M. – Lakatos, E. – Kovács, A.J. (2005):** Nyers és fogyasztói tej zsírtartalmának meghatározása mikrohullám és konvektív szárítási eljárás során. Lippay János – Ormos Imre – Vas Károly tudományos ülésszak. 2005. 10. 19-21. Proceedings. pp. 272.

12. **Neményi, M. – Lakatos, E. – Kovács, A.J. (2006):** Mikrohullám kezelés használata nyers és fogyasztói tej zsírtartalmának meghatározására. MTA-AMB, 30. Kutatási-Fejlesztési Tanácskozás, Gödöllő, 2006. Proceedings, szerk. Dr. Fenyvesi László pp. 10.

13. **Lakatos, E. – Kovács, A.J. – Neményi, M. (2006):** Homogén mikrohullámú tér kialakítása. VII. Nemzetközi Élelmiszertudományi Konferencia. 2006. április 20. Szeged.