

PhD Thesis

APPLICATION OF RAPID METHODS FOR MONITORING QUALITY CHANGES OF PORK MEAT

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Introduction:

There is general need for less labour intensive, rapid and reliable methods for monitoring microbiological quality in the food chain to identify hygienic and safety problems more rapidly, so that corrective actions can be taken.

In relation to meat and meat products, issues both of microbiological safety and shelf-life are concerned, and temperature is one of the major factors in them.

Aim of work:

My intention was to study the potential for assessing a relatively rapid, physical and automated screening technique for simultaneous estimation of microbiological status of large numbers of samples for monitoring spoilage bacteria on chilled pork cutlets.

My aim was to study the utility of conductometric method to follow bacteriological deterioration of pork during storage.

My objective was to study the utility of chemo sensor array signals of head-space volatiles of aerobically packed pork cutlets as a non-invasive technique to estimate development of bacterial spoilage at various storage temperatures.

I intended to study the utility of near infrared spectroscopy as a non-invasive technique to follow bacterial deterioration of pork during storage.

Materials and methods:

Materials:

 Slices of fresh cutlets of de-boned pork were transferred to sterile petri-dishes and experimental batches of these samples were stored under aerobic conditions at 4, 8, and 12 °C temperatures, respectively. 2. Minced pork samples were transferred to sterile petri-dishes and these samples were stored under aerobic conditions at 4°C temperature.

Methods:

- 1. Bacteriological quality, namely standard total aerobic plate counts (TAPC) and selectively estimated *Pseudomonas* (PS) counts of pork slices were determined by pour-plate method. Besides *Pseudomonas* counts, *Brochotrix thermosphacta* and *Lactobacillus* counts were determined in the minced pork.
- 2. A Malthus Microbiological Analyzer was used in the examinations of pork slices.
- 3. A NST3320 type electronic nose was used in the examination of volatile compounds produced by bacterial spoilage of pork slices.
- 4. Near infrared spectra were recorded by a SPECTRALYZER 1025 PMC type NIR spectrometer in case of sliced pork, and the minced pork samples were measured by a MetriNir 10-17 type NIR spectrometer.
- 5. PCA, PLS, CDA chemometric methods were used for evaluating the analysed neasured data.

Results:

Microbiology:

The aerobic and moderately aerobic spoilage flora of fresh sliced and minced pork stored at chill temperature was dominated by psychrotrophic aerobic, Gram-negative *Pseudomonas* spp.

GSP agar seemed to be better for our purpose than Cetrimide agar because microscopic checking of the colonies showed that the Cetrimid agar inhibited also some part of the *Pseudomonas* population. In minced pork, *Brochotrix thermosphacta* and *Lactobcillus* counts were determined, too. Neither of them could dominate under the storage conditions applied.

Automatic conductometry:

The conductometric method seemed to be suitable to assess within 8 hours whether a sample of pork meat had cell counts higher or less than 10^7 CFU g⁻¹ of psychrotrophic spoilage bacteria, whereas at least 72 h were needed to obtain the same information by colony counting techniques.

The relative rapidity and its ability to process large numbers of samples makes conductometry a suitable method for monitoring microbial state and freshness of meat.

Electronic nose:

Considering the correlation between viable cell counts versus individual signal responses, linear correlation was found over r>0.70 between EN responses and TAPC.

The elimination of 14 insensitive sensors, having poor information in this special application, hardly modified the efficiency parameters of the computed models.

Regarding our observations that only a few individual sensors (9 sensors) relatively high correlations with the reference data, it could be expect that construction of a cheaper, single purpose instrument with only a few sensors with significant discrimination power can be proposed for the spoilage assessment of meats.

The experiments show that electronic nose measurements may be able to reveal changes in the head-space volatiles of aerobically packed meat. The electronic nose has the advantage of being rapid, non-destructive and non-contact instrumental testing.

Near infrared spectroscopy:

In samples with bacterial counts of 10⁶ CFU/g no organoleptic changes could yet be experienced. NIR spectroscopy indicated the quality changes already at this microbial state in the product.

Sample preparation time became minimal, this way examinations can even be performed on the spot.

These preliminary results indicate the potential of utilizing near infrared diffuse reflectance spectroscopy in combination with multivariate statistical methods to rapidly monitor loss of freshness and detect bacterial spoilage of meat samples before colinearity microbial changes become apparent.

Conclusion:

The objectives of my Ph.D. thesis were aimed to evaluate the opportunity of developing a rapid and automatic system.

However, the applied methods are correlative and require calibration, the observation period is shorter than in case of the standard microbiological methods, also larger numbers of samples can be tested.

The application of statistical methods is critical for understanding the results, therefore the instrumental examinations highlighted in my thesis are applicable appropriately only together with the chemometric methods.

My investigations support that rapid instrumental methods applied and described in my thesis are suitable for tracking the changes that occur during the storage of sliced and minced pork meat.

Suggestions:

As the electronic nose was less sensitive than near infrared spectroscopy, the electronic nose is applicable rather to describing the development of spoilage, while NIR technology can provide opportunity for following up/monitoring the bacterial count.

In the framework of my PhD thesis I had the opportunity for orientation experiments with instrumental examinations. The results of the electronic nose and NIR technology experiments are promising in the perspective that routine control processes can be done/performed by these methods in numerous areas of the food industry. Since the principle of the rapid methods is different, the reliability of the examinations can be increased by the parallel application of multiple methods.

It is indispensable for the application in practice and for increasing the statistical reliability to have more similar experiments done so later on portable/online tools could be developed that are not included in my thesis.

New scientific thesis:

- 1. My experiments have proven that spoilage of chilled sliced and minced pork meat is mainly caused by the Pseudomonas spp. both under aerobic and moderately aerobic storage conditions, therefore the number of colonies formed on selective medium shows stronger correlation with the detection time than the total aerobic count does. Microbiological changes occurring in the pork meat during storage at 4 and 8°C are similar, while spoilage at 12°C is different, caused by more heterogenic micro biota.
- 2. The results of my experiments proved that compared to the standard microbiological pour plate method, which takes 72 hours, Malthus instrument is suitable to assess the limit of microbiological acceptability (10⁷ CFU g⁻¹) of sliced pork meat within 8 hours while 12-13 hours are needed to determine the hygienic level required by the EU regulation (10⁴ CFU/g).
- 3. I proved with my experiments that in case of pork meat the electronic nose is applicable/suitable for the detection of significant growth of spoilage bacteria, long before the spoilage could be detected by sensory organs. In case of sliced pork meat 9 sensors out of 23 of the NST 3320 apparatus are sufficient for this purpose.
- 4. I established that in case of sliced and minced pork meat near infrared spectroscopy is applicable to the quick and non destructive detection of bacterial spoilage, long before it could be detected by sensory organs.

List of publication

Articles in Journal

IF Articles

- K. HORVÁTH, É. ANDRÁSSY, M. KORBÁSZ, J. FARKAS, (2007): Using automatic conductometry for monitoring spoilage bacteria on chilled pork cutlets, Acta Alimentaria vol.36 (2) pp.283-291
- 2. **K. HORVÁTH**, ZS. SEREGÉLY, I. DALMADI, É. ANDRÁSSY, J. FARKAS, (2008): A prelimary study using near infrared spectroscopy to evaluate freshness and detect spoilage in sliced pork meat, Acta Alimentaria vol 37 (1) pp.93-102
- 3. L. FRIEDRICH, I. SÍRÓ, I. DALMADI, **K. HORVÁTH**, R. ÁGOSTON, CS. BALLA (2008): Influence of various preservatives on the quality of minced beef under modified atmosphere at chilled storage Meat Science vol. 79 pp.332-343

Articles without IF

- 1. OZSVÁTH P., NÉMETH CS., FRIEDRICH L., NÉMETH Z., ZEKE I., **HORVÁTH K**. PÁSZTORNÉ H. K., BALLA Cs. (2009): Héj nélküli, főtt egész tojások kiskereskedelmi hűtve tárolási lehetőségeinek vizsgálata, *Élelmezési Ipar*, 63 (4), 115-118. p.
- 2. NÉMETH CS., **HORVÁTH K.**, FRIEDRICH L., PÁSZTORNÉ H. K., ZEKE I., BALLA CS. (2009): A tojásfehérjelé, a tojássárgájalé, és a teljes tojáslé hőérzékenységének vizsgálata, *Baromfi ágazat*, 1, 72-74
- 3. **K. M. HORVÁTH**, ZS. SEREGÉLY, I. DALMADI, É. ANDRÁSSY, J. FARKAS, (2007): Estimation of bacteriological spoilage of pork cutlets by electronic nose Acta Microbiologica et Immunologica Hungarica 54 (2) pp. 179-194
- HORVÁTH K., SEREGÉLY, ZS., DALMADI, I., ANDRÁSSY, É., FARKAS, J., FRIEDRICH, L. (2007): Szeletelt sertéshús bakteriológiai minőségének becslése gyors műszeres módszerekkel, Élelmezési Ipar 6 (11) pp.346-349

Conference Proceedings

Hungarian (abstract)

- HORVÁTH, K., DALMADI, I., ANDRÁSSY, É., FARKAS, J. (2008): Chemometric evaluation of near infrared spectroscopy measurements for bacteriological spoilage assessment of chilled boneless slice of pork meat, Magyar Mikrobiológiai Társaság 2008. évi Nagygyűlése, Keszthely, 2008.október 15-17. Összefoglaló pp. 30
- MÁRTA, D., HORVÁTH, K., BELÁK, Á., ANDRÁSSY, É. FARKAS, J., MARÁZ, A., (2007): Hűtve tárolt sertéshús romlási folyamatának modellezése és a pszeudomonasz populációk vizsgálata molekuláris módszerekkel. Hungalimentaria 2007 "Szakemberek a biztonságosabb élelmiszerláncért", Budapest, 2007. október 25-26., pp. 35.
- 3. **HORVÁTH K.**, FRIEDRICH, L., KORBÁSZ, M., ANDRÁSSY, É., BECZNER, J., FARKAS, J. (2007): Baktériumok sertéshúson szaporodásának nyomon követése automatikus impedimetriával. KÉKI/ÉKB/MÉTE 2006. április 27-én tartott 324. tudományos kollokvium előadásának kivonata. Élelmezési Ipar, 61 (6) pp. 175
- HORVÁTH K., SEREGÉLY, ZS., DALMADI, I., ANDRÁSSY, É., FARKAS, J., FRIEDRICH, L. (2007): Baktériumos romlás becslése szeletelt sertés húson gyors műszeres módszerekkel. Lippay János Ormos Imre Vas Károly Tudományos Ülésszak, Budapest, 2007. november 7-8., Összefoglalók, pp.58-59.
- HORVÁTH K., SEREGÉLY, ZS., ANDRÁSSY, É., DALMADI, I., FARKAS, J. (2007): Kísérletek közeli infravörös spektroszkópia (NIR) alkalmazására szeletelt sertés hús frissességének és romlásának gyors becslésére. Centenáriumi Vegyészkonferencia, Sopron, 2007. május 29-június 1., Összefoglalók, pp. 246
- 6. K. HORVÁTH, I. DALMADI, M. KORBÁSZ, ZS. SEREGÉLY, É. ANDRÁSSY, J. FARKAS, (2006): Application of rapid instrumental methods for monitoring bacteriological spoilage of meat, Acta microbiologica et immunologica Hungarica, Annual Meeting of the Hungarian Society for Microbiology, 2006. október 18-20., pp.277-278
- 7. **HORVÁTH K**,, FARKAS J., BECZNER J. (2005) : *Listeria monocytogenes* szaporodásának becslése ComBase program segítségével, Lippay János Ormos Imre Vas Károly Tudományos Ülésszak, Budapest, 2005. október 19-20., Összefoglaló pp.146-147
- 8. **HORVÁTH K**. (2005): A ComBase program gyakorlati használata az élelmiszerforgalmazásban, Országos Tudományos Diákköri Konferencia, Szarvas, 2005. március 31-április 2. Összefoglaló pp.134
- 9. BECZNER J., FARKAS J., **HORVÁTH K**., (2004): Használati tapasztalatok nemzetközi prediktív mikrobiológiai szoftverekkel, Magyar Mikrobiológiai Társaság 2004. évi Nagygyűlése, Keszthely, 2004. október 7-9 Összefoglaló pp. 9

Hungarian (full paper)

 CS. BALLA, L. FRIEDRICH, I. ZEKE, K. HORVÁTH, CS. FARKAS, R. ÁGOSTON (2008): Temperature- monitoring of refrigerated display cabinets in supermarket in time and space with thermocamera and RFID technology *Cold Chain- Managenet, June 2-3 Bonn*, pp.298-303

International Conference (abstract)

- 2. MÁRTA, D., **HORVÁTH, K**., KRASCSENICS, K., FARKAS, J., MARÁZ, A.(2008): Detection and identification of food spoiling Pseudomonas species during refrigerated storage of pork cutlet. Programme and Abstract Book, FoodMicro 2008, The 21st International ICFMH Symposium Aberdeen, U.K., September 1-4. P128, pp. 254.
- CS. BALLA, L. FRIEDRICH., I. ZEKE, K. HORVÁTH, CS. FARKAS, R. ÁGOSTON (2008): Temperature-monitoring of refrigerated display cabinets in supermarkets in time and space with thermocamera and RFID technology, Cold Chain-Management 3rd International Workshop, June 2-3., Bonn ISBN-978-3-9812345-0-3 pp. 298-303
- 4. K. PÁSZTOR-HUSZÁR, T. STRIXNER, **K. HORVÁTH** (2008) Spectrofluorometric detection of changes in milk as a result of heat or HPP treatment, 15-17 May 2008, Cavtat, Croatia pp. 192
- MÁRTA, D., HORVÁTH, K., BELÁK, Á., ANDRÁSSY, É., FARKAS, J., MARÁZ, A (2007):Refrigerated storage of pork cutlet: monitoring and molecular identification of food spoiling, Power of Microbes in Industry and Environment, , September 19-22, Zadar, Croatia, pp. 121
- 6. K. HORVÁTH, D. MÁRTA, É. ANDRÁSSY, SZ. TYAHUR, J. FARKAS, A. MARÁZ, (2007): Studie on the development of specific bacterial spoilage biota in chilled minced pork meat, Acta microbiologica et Immunologica Hungarica 15 th International Congress of the Hungarian Society for Microbiology, 2007 July 18-20, pp.:50
- 7. E. TUBOLY, **K. HORVÁTH**, CS. BALLA, (2006): Monitoring the temperature of chilled meat products in a hypermarket chain by using infrared thermo camera, CeFood konferencia 22-24 May 2006, Sofia, Bulgaria, pp.126
- 8. DALMADI, ZS. SEREGÉLY, **K. HORVÁTH**, CS. BALLA, (2006): Monitoring the effect of crumb on sunflower oil drying frying by NIR spectroscopy and chemosensor array, CeFood konferencia, 22-24 May 2006, Sofia, Bulgaria, pp.125
- K. HORVÁTH, É. ANDRÁSSY, M. KORBÁSZ, J. BECZNER, J. FARKAS, L. FRIEDRICH, (2006): The Use of automatic impedimetry for monitoring spoilage on chilled pork cutlets, Food Micro 2006 August 29- September 2 2006, pp. 540
- 10. J. FARKAS, ZS. SEREGÉLY, I. DALMADI, **K. HORVÁTH**, É. ANDRÁSSY, L. FRIEDRICH, (2006): Chemometric evaluation of electronic nose measurements for bacteriological spoilage

- assessment of refrigerated pork meat, Bologna, Italy, Food Micro 2006, August 29- September 2 2006, pp. 563
- 11. KAFFKA, K., DALMADI, I., **HORVÁTH, K.** (2007): Classifying paprika seeds according to variety and harvest year using their NIR spectra *The 13th International Conference on Near Infrared Spectroscopy (13th ICNIRS), Umeå-Vasa, Sweden & Finland 15-21 June 2007, NIR Abstracts: C-52 276, on-line: http://www.nir2007.com/abstracts/system/poster_c/C-53_276.pdf*

Book, Book Chapters, Textbook

Hungarian

- FARKAS J., HORVÁTH K., (2006): Kockázatbecslés, jellemzés baktérium szaporodási adatbank és szoftverek használatával In: Pásztorné Huszár K., Kiss I. (szerk): Minőség-kímélő élelmszertechnológiák és élelmiszer-biztonság, BCE ÉTK és Mezőgazda Kiadó, Budapest, pp.156-159
- FARKAS J., HORVÁTH K., (2007): Az élelmiszer- biztonsági kockázatelemzés alapismeretei In: Balla Csaba, Síró István (szerk.): Élelmiszer-biztonság és –minőség I. Alapismeretek, Mezőgazda Kiadó, Budapest, pp. 289-301

<u>Citation</u>

- **K. M. HORVÁTH**, ZS. SEREGÉLY, I. DALMADI, É. ANDRÁSSY, J. FARKAS, (2007): Estimation of bacteriological spoilage of pork cutlets by electronic nose, Acta Microbiologica et Immunologica Hungarica 54 (2) pp. 179-194
 - DALMADI I., SEREGÉLY ZS., KAFFKA K., FARKAS J. (2007): Néhány többváltozós kemometriai módszeralkalmazása műszeres analitikai vizsgálatok értékelésére, Élelmiszervizsgálati közlemény, 53 (4), pp. 222-238
- L. FRIEDRICH, I. SÍRÓ, I. DALMADI, **K. HORVÁTH**, R. ÁGOSTON, CS. BALLA (2008): Influence of various preservatives on the quality of minced beef under modified atmosphere at chilled storage Meat Science vol. 79 pp.332-343
 - 1. MOHAMED B., JAMILAH K.A., ABBAS AND R. ABDUL RAHMAN (2008): A review on some organic acids additives as shelf life extenders of fresh beef cuts, American Journal of Agricultural and Biological Sciences, 3 (3), pp. 566-574
 - 2. KENETH W. MCMILLIN (2008): Where is MAP going? A review and future potential of modified atmosphere packaging meat, Meat Science, 80, pp.43-65