APPLICATION OF NEAR INFRARED SPECTROSCOPY FOR RAPID NONINVASIVE DETECTION OF *LISTERIA MONOCYTO-GENES*, *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* GROWTH IN FOODS

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Summary

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Meat and cheese samples, artificially contaminated by three bacterial species: *Listeria monocytogenes, Escherichia coli* and *Staphylococcus aureus* were investigated by both microbiology and near infrared spectroscopy (NIRS). The incubation of samples at 8 °C for 14 days did not result in significant increase in the number of inoculated microorganisms. During the entire experimental period, microbial counts remained within 10^1–10^3 CFU/g product. Regardless of the low bacterial contamination, NIRS analysis has successfully distinguished the contaminated samples by the SIMCA model and exhibited specific spectral differences that could be used to differentiate the specific classes according to the bacterial species.

Key words: cheese, foodborne pathogens, food safety near infrared spectroscopy, sausage

INTRODUCTION

Listeria monocytogenes, *Escherichia coli* and *Staphylococcus aureus* are among the most important pathogens causing foodborne diseases of public health relevance. The presence of viable bacterial cells, toxic metabolites or products from their reproduction in foodstuffs or ready-to-consume foods is a health hazard from the point of view of the safe food production concept (Hathaway, 1995; Evers *et al.*, 2002). Regulations (EC) 2073/2005 and (EC) 1441/2007 specify the control on *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus* and set up the norms for their presence in foods (Anonymous, 2005; 2007). The USA

strategy for higher food safety introduces the zero-tolerance policy with regard to *L. monocytogenes* by defining its presence in ready-to-consume foods as a hazard for consumer's health. At the same time, the monitoring has shown that about 5% of ready to consume foods are contaminated with *L. monocytogenes* (Hitchins, 1996; Levine *et al.*, 2001). Cases of mass or sporadic food poisonings with *Escherichia coli* and *Staphylococcus aureus* are reported (Schmid *et al.*, 2007; Hart & Smith, 2009; Kitamoto *et al.*, 2009).

The routine detection of bacteria in foods consists of initial cultivation on specific culture media followed by bio-

chemical or serological identification. This protocol is related to laboratory costs and takes at least 48–72 hours, while express immunological and genetic methods are still very expensive.

Near infrared spectroscopy (NIRS) is a rapidly developing non-destructive low-cost method with multiple applications in the analysis of meat and dairy products (Lucas *et al.*, 2008; Prieto *et al.*, 2009). It is based upon the absorption of C-H, O-H and N-H molecular bonds of food ingredients in the wavelength range from 750 to 2500 nm (Sandorfy *et al.*, 2007). The information from spectra is obtained by high-potential mathematical methods for qualitative analysis and quantitation.

Lin *et al.* (2004) used NIRS to investigate the spoilage of poultry meat and concluded that this spectrum could be used for rapid quantitative determination of microbial contamination rates. Suthilik *et al.* (2008) have used NIRS in the 700–1100 nm range to determine the bacterial contamination of shredder cabbage.

The purpose of this experiment was to determine the potential of NIRS to detect low-rate microbial contamination of samples from meat and dairy products with *Listeria monocytogenes*, *Escherichia coli* or *Staphylococcus aureus*.

MATERIALS AND METHODS

Sample preparation and contamination

The experiment was performed with two types of ready to eat foods purchased from a retail store – Bulgarian yellow cheese and cooked beef sausage. Under strict observance of aseptics, slices of 3–4 mm thickness were made and put in sterile 38-mm Petri dishes. For each product, 48 Petri dishes were prepared, divided in 3 groups of 16 (groups I, II and III). For contamination of Petri dishes from groups I, II and III, 0.1 mL from a serial dilution of bacterial culture containing up to 100 CFU (viable cells) of *Listeria monocytogenes*, *Escherichia coli* or *Staphylococcus aureus*, respectively were used. The amount of 0.1 mL is spread evenly on sample surface with a sterile glass rod. After the contamination, samples were packed by pairs in individual vacuum envelopes and put in a cooling thermostat chamber (TLSO 80/80, Etna) at 8.0 ± 0.3 °C for 14 days. The pH of foodstuffs was determined with a pH-meter (PB-11, Sartorius), and water activity (aW) – with HygroLab 2, Rotronic.

Bacterial suspension

Samples were contaminated with reference strains of *Listeria monocytogenes* NCTC 11994, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. Broth cultures of each species were prepared in Triptone Soya Broth with Yeast Extract (TSBYE) (Merck) by incubation at 30 °C for 18 h. The obtained bacterial culture contained about 10^8 CFU/mL of *L. monocytogenes* and 10^9 CFU/mL of each *E. coli* and *S. aureus*. From the initial suspensions, serial dilutions of 10^{-4} CFU/mL (*L. monocytogenes)* and 10^{-5} (*E. coli* and *S. aureus*) were prepared in tubes containing 9 mL MRD diluent. About 100 bacterial cells were enumerated in 0.1 mL. To determine the exact number of microorganisms, 0.1 mL of dilutions were inoculated on ALOA (Merck), TBX (Merck) or Baird Parker (Merck) agars.

Microbiological analysis

Specimens from the three groups of prepared samples were obtained at 48-hour intervals for spectral and microbiological testing. The first (zero) determination was

done 30 min after inoculation, a time sufficient for attachment and adaptation of microorganisms to the matrix surface. Each measurement included 2 specimens from each group. The spectral analysis was run first and immediately afterwards, the microbial counts were determined by classical inoculations on ALOA (Merck), TBX (Merck) or Baird Parker (Merck) agars according to the bacterial species.

Spectral analysis

Diffuse reflectance spectra of studied samples were obtained on a scanning mobile spectrophotometer NIRGUN® (FANTEC, Japan), working in the near infrared spectrum from 600 to 1100 nm (at 2 nm steps). Individual spectra were obtained from 5 different sites from the sample surface or total of 10 sites from both Petri dishes for each studied microorganism.

The obtained spectral data were processed with Pirouette 2.0 software (Infometrics, Inc., Woodinville, WA, USA), that used data further for classification by the Soft Independent Modeling of Class Analogy (SIMCA) method. This method, divided samples into classes on the basis of a preselected parameter. In this study, samples were divided according the microbial species used for contamination. For each class, the recognition model was designed on the basis of main components of spectral data of samples from the respective class. The spectral information of SIMCA models was analysed to determine the information relevant for classification of samples. The modeling power parameter for each class was investigated. The higher the modeling power for a given wavelength, the higher the relevance of the absorption at this wavelength for the respective class' model was.

RESULTS

Microbiological analysis

Working dilutions of broth cultures used to contaminate samples of dairy and meat products contained in average 2.3×10^2/ 0.1 mL CFU *L. monocytogenes*, 1.1×10^2 CFU/0.1 mL *E. coli* and 1.3×10^2 CFU/0.1 mL *S. aureus*. The pH values of Bulgarian yellow cheese and cooked beef sausage were 6.61 and 5.48. The respective water activity values were 0.942 and 0.958.

The results from the microbiological analysis of contaminated samples are pre-

Table 1. Total viable cell counts of *Listeria monocytogenes, Escherichia coli* and *Staphylococcus aureus* in contaminated Bulgarian yellow cheese and cooked beef sausage samples stored at 8 °C.

Hour	Bulgarian yellow cheese, CFU/g			Cooked beef sausage, CFU/g		
	L. monocytogenes	*E.coli*	*S.aureus*	*L. monocytogenes*	*E.coli*	*S.aureus*
0	1.0×10^1	0.9×10^1	0.9×10^1	1.0×10^1	0.7×10^1	1.4×10^1
48	1.5×10^1	1.0×10^1	0.4×10^1	5.5×10^1	1.0×10^1	1.0×10^1
96	1.0×10^1	1.0×10^1	1.0×10^1	1.0×10^1	2.0×10^1	1.1×10^1
144	5.5×10^1	1.0×10^1	1.0×10^1	5.5×10^1	1.5×10^1	1.5×10^1
192	5.5×10^1	1.0×10^1	1.0×10^1	5.5×10^1	1.5×10^1	1.5×10^1
240	5.5×10^1	1.0×10^1	1.0×10^1	5.5×10^1	1.0×10^1	1.0×10^1
288	1.1×10^2	1.0×10^1	1.0×10^1	1.1×10^2	1.0×10^1	1.2×10^1
336	1.0×10^2	1.0×10^1	1.0×10^1	1.2×10^2	1.0×10^1	1.0×10^1

Table 2. Results from the SIMCA classification of spectral data of Bulgarian yellow cheese and cooked beef sausage samples contaminated with *Listeria monocytogenes, Escherichia coli* and *Staphylococcus aureus*

| Class	n	Correctly classified	Incorrectly classified
Bulgarian yellow cheese			
L.monocytogenes	80	79 (98.8%)	1 (1.2%)
E.coli	80	77 (96.3%)	3 (3.7%)
S.aureus	80	79 (98.8%)	1 (1.2%)
Cooked beef sausage			
L.monocytogenes	80	79 (98.8%)	1 (1.2%)
E.coli	80	79 (98.8%)	1 (1.2%)
S.aureus	80	80 (100%)	0 (0.0%)
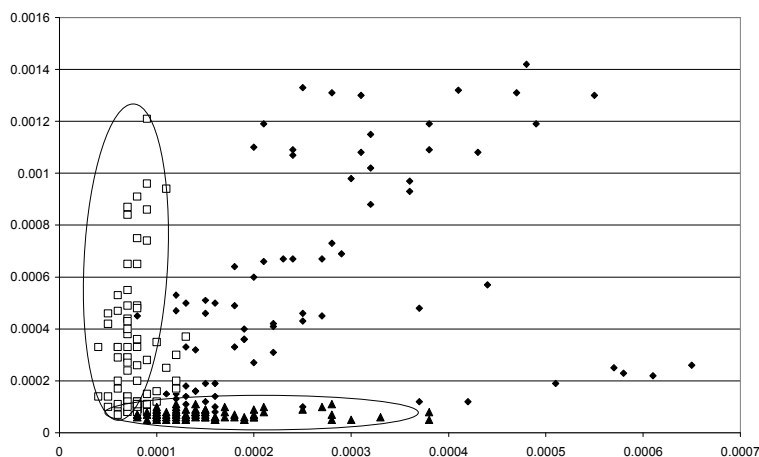
Fig. 1. Distribution of spectral characteristics of Bulgarian yellow cheese samples, contaminated with *Listeria monocytogenes* (♦)*, Escherichia coli* (□) and *Staphylococcus aureus* (▲).

sented in Table 1. The average viable cell counts in Bulgarian yellow cheese at experiment's beginning were 1×10^1 CFU/g, 0.9×10^1 CFU/g and 0.9×10^1 CFU/g for *L. monocytogenes*, *E. coli* and *S. aureus,* respectively, whereas those of cooked beef sausage were 1×10^1, 0.7×10^1 and 1.4×10^1 CFU/g. The counts of *L. monocytogenes* varied between 1×10^1 in the beginning and 1.1×10^2 at the end of the study, those of E. *coli:* from 0.7×10^1 to 2.0×10^1 and of *Staphylococcus aureus* – from 0.4×10^1 to 1.5×10^1.

Spectral analysis

The applied SIMCA method distributed the samples in three classes – the first consisting of samples contaminated with *L. monocytogenes*, the second – with *E. coli* and the third – with *S. aureus*. The different classes and the extent of bacterial contamination were determined on the
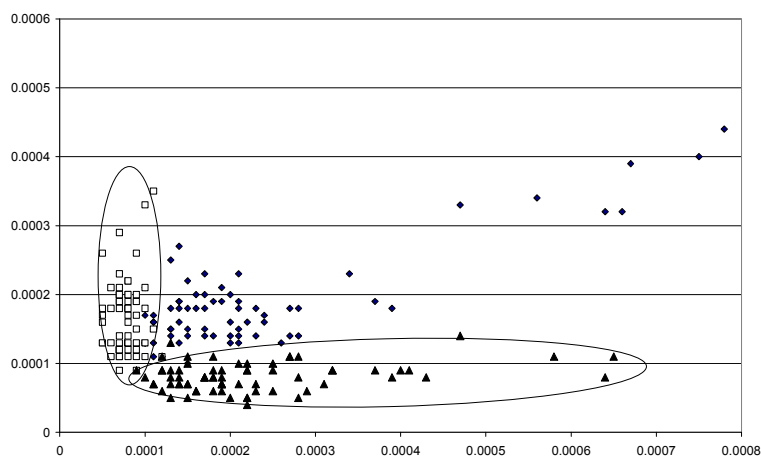
Fig. 2. Distribution of spectral characteristics of cooked beef sausage samples, contaminated with *Listeria monocytogenes* (♦)*, Escherichia coli* (□) and *Staphylococcus aureus* (▲).
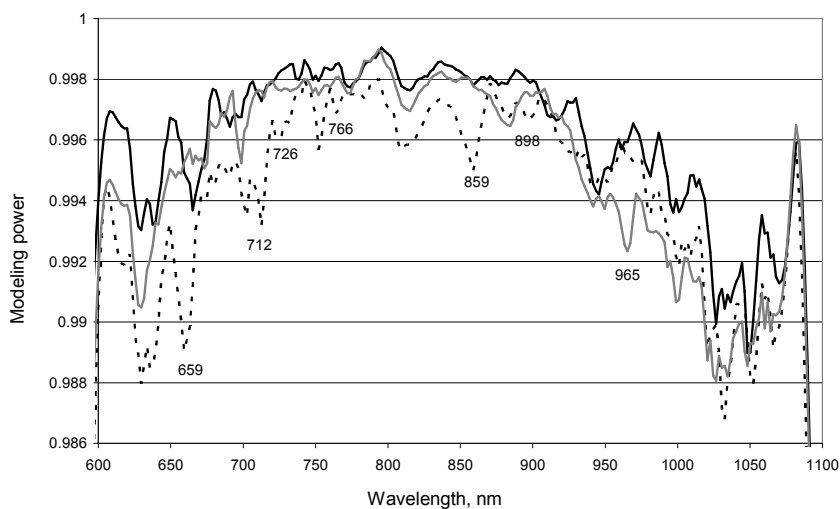
Fig. 3. Modeling power vs wavelengths plots for the three classes of Bulgarian yellow cheese samples: *Listeria monocytogenes* (black line)*, Escherichia coli* (grey line) and *Staphylococcus aureus* (dotted line).

basis of microbiological analysis results. The results from the SIMCA classification of cheese and sausage samples are presented in Table 2. The highest precision of recognition (98.8%) was exhibited for samples contaminated with *L. monocyto-*

genes and *S. aureus*. From samples contaminated with *E. coli*, 3 were incorrectly classified, hence the lower precision of the model for this bacterial species (96.3%).

Similar data were obtained for sausage samples: 98.8% of samples contaminated

with *L. monocytogenes* and *E. coli* were correctly classified. All samples with *S. aureus* were properly recognized (100%). The discrimination and 3D distribution of spectral data of the different classes of Bulgarian yellow cheese and cooked beef sausage samples are presented on Fig. 1 and 2, and Fig. 3 depicts the relevance of spectral data for each wavelength in the respective model for cheese samples.

DISCUSSION

Microbiological analysis of studied samples showed that after contamination of samples with the respective working dilution of broth culture of *E. coli* and *S. aureus,* the microbial counts did not increased significantly (more than tenfold). Notermans *et al.* (2006) have shown that *S. aureus* did not replicate at water activity values lower than 0.85, pH under 4.3 and temperatures <8 ºC. For *E. coli*, the respective minimum values are 0.95, pH 4.4 and 7 ºC (Bell & Kyriakides, 1998). In a number of microorganisms, including those used in our research, the growth and normal development of bacterial cells are slowed down or stop at pH<4.5 and aW<0.88 (Cervenka *et al.*, 2004; Giaouris *et al.*, 2005; Notermans *et al.*, 2006). In this experiment, water activity and pH were within the optimal range for bacterial growth but the temperature of 8 ºC was adverse for used bacterial species. Samples contaminated with *L. monocytogenes* exhibited a low (tenfold) increase in the number of bacteria up to 1.2×10^2 CFU/g product. The storage conditions of foodstuffs and ready-to-eat foods in industrial or retail settings require maintaining a temperature from 2 to 4 ºC. Often, due to production or technical flaws, the storage temperature passes over the critical value of 6–8 ºC and creates preconditions for

replication of bacterial flora in the product reducing the product safety (Evers *et al.*, 2002), especially with regard to dangerous species as *L. monocytogenes, E. coli* and *S. aureus.*

The high precision of the SIMCA model for recognition of the different microbial species in studied samples showed a relationship between NIR spectra of samples and the contamination rates with *L. monocytogenes*, *E. coli* and *S. aureus*. The accuracy of classifications was determined for low contamination rates (about 10 bacterial cells in 1 g product). The number of correctly classified samples was similar to what was reported by Lin *et al.* (2004) in poultry meat, where the correlation coefficient between spectral data and the number of CFU was 0.91, with error of determination 0.48 log CFU.g^{-1}.

The spectral information of SIMCA models was analysed to establish the information, relevant for sample classification and the relationship of absorption at a given wavelength with specific changes in sample content due to microbial presence and replication. The modeling power vs wavelength plots presented on Fig. 3 showed that the higher modeling power values were indicative of higher relevance of spectral data for a given wavelength. The figure shows marked difference between modeling powers of samples from the three tested classes due to differences in spectra caused by the different bacterial species. The most significant differences were observed at wavelengths of 659, 712, 726, 766, 859, 898 and 965 nm, the absorption being due mainly to CH and OH groups of protein and carbohydrate components (Sandorfy *et al.*, 2007). Bacteria, pathogens or not, use mainly protein and carbohydrate from substrates to maintain their vital functions or for replication

and bacterial biomass augmentation. According to our data, the development of bacteria in Bulgaria cheese samples was altered in a specific manner that could be spectrally recognized.

Similar results were obtained in SIMCA classification of cooked beef sausage samples.

In conclusion, in spite of the negligible bacterial contamination (10 CFU/g product) with *L. monocytogenes, E. coli* and *S. aureus,* contaminated samples exhibited clear differences in NIR spectra depending on the respective contaminating foodborne pathogen. Therefore, NIRS is potentially useful for rapid and noninvasive detection of bacterial growth in animal foodstuffs.

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REFERENCES

Anonymous, 2005. Commission Regulation (EC) No 2073/2005 Microbiological criteria for foodstuffs. *Official Journal of the European Union,* L 338, 1–26.

Anonymous, 2007. Commission Regulation (EC) 1441/2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union* L 322, 12–29.

Bell, C. & A. Kyriakides, 1998. *E. coli:* A practical approach to the organism and its control in foods. Blackie Academic and Professional, New York, New York, USA, pp. 208.

Cervenka, L., Z. Malíková, I. Zachová & J. Vytrasová, 2004. The effect of acetic acid, citric acid, and trisodium citrate in combination with different levels of water activity on the growth of *Arcobacter butzleri* in

culture. *Folia Microbiologica (Praha)*, **49**, 8–12.

Evers, E. G., M. J. Nauta, A. H. Havelaar & A. M. Henken, 2002. Quantitative risk assessment of food borne pathogens – a modeling approach. In: *New Approaches to Food-Safety Economics. Proceedings of the Frontis workshop on new approaches to food-safety economics,* Wageningen, the Netherlands 14–17 April 2002, pp. 35–45.

Giaouris, E., N. Chorianopoulos & G. Nychas, 2005. Effect of temperature, pH, and water activity on biofilm formation by *Salmonella enterica enteritidis* PT4 on stainless steel surfaces as indicated by the bead vortexing method and conductance measurements. *Journal of Food Protection,* **68**, 2149–2154.

Hart, J. & G. Smith, 2009. Verocytotoxin-producing *Escherichia coli* O157 outbreak in Wrexham, North Wales, July 2009. *Eurosurveillance,* **14**, 193–200, http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19300 (date last accessed July 06 2011).

Hathaway, S., 1995. Harmonization of international requirements under HACCP based food control systems. *Food Control*, **6**, 267–276.

Hitchins, A. D., 1996. Assessment of alimentary exposure of *Listeria monocytogenes*. *International Journal of Food Microbiology*, **30**, 71–85.

Kitamoto, M, K. Kito, Y. Niimi, S. Shoda, A. Takamura, T. Hiramatsu, T. Akashi, Y. Yokoi, H. Hirano, M. Hosokawa, A. Yamamoto, N. Agata & N. Hamajima. 2009. Food poisoning by *Staphylococcus aureus* at a university festival. *Japanese Journal of Infectious Diseases,* **62**, 242–243.

Levine, P., B. Rose, S. Green, G. Ransom & W. Hill, 2001. Pathogen testing of ready-to-eat meat and poultry products collected at federally inspected establishments in the United States, 1990–1999. *Journal of Food Protection,* **64**, 1188–1193.

Lin, M., M. Al-Holy, M. Mousavi-Hesary, H. Al-Qadiri, A. G. Cavinato & B. A. Rasco,

2004. Rapid and quantitative detection of the microbial spoilage in chicken meat by diffuse reflectance spectroscopy (600–1100 nm). *Letters in Applied Microbiology*, **39**, 148–155.

Lucas, A., D. Andueza, E. Rock & B. Martin. 2008. Prediction of dry matter, fat, pH, vitamins, minerals, carotenoids, total antioxidant capacity, and color in fresh and freeze-dried cheeses by visible-near-infrared reflectance spectroscopy. *Journal of Agricultural and Food Chemistry,* **56**, 6801–6808.

Notermans, S. & C. J. Neuvelman, 2006. Combined effect of water activity, pH and sub-optimal temperature on growth and enterotoxin production of *Staphylococcus aureus*. *Journal of Food Science*, **48**, 1832–1835.

Prieto, N., R. Roehe, P. Lavín, G. Batten & S. Andrés. 2009. Application of near infrared reflectance spectroscopy to predict meat and meat products quality: A review. *Meat Science*, **83**, 175–186.

Sandorfy, C., R. Buchet & G. Lachenal, 2007. Principles in molecular vibrations for near-infrared spectroscopy. In: *Near Infrared Spectroscopy in Food science and Technology*, John Wiley & Sons, Inc. Canada, pp. 11–48.

Schmid, D., E. Gschiel, M. Mann, S. Huhulescu, W. Ruppitsch, G. Bohm, J. Pichler, I. Lederer, G. Hoger, S. Heuberger & F. Allerberger, 2007. Outbreak of acute gastroenteritis in an Austrian boarding school, September 2006. *Eurosurveillance*, **12**, pp. 224, http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=692 (date last accessed July 06 2011).

Suthilik, P., S. Saranwong, S. Kawano, S. Numthuam & T. Satake. 2008. Possibility of using near infrared spectroscopy for evaluation of bacteria contamination in shredder cabbage. *International Journal of Food Science and Technology*, **43**, 160–165.

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