Application of near-infrared spectroscopy for detection of bacterial contamination in food

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Introduction

Food safety strategy and measurement for reducing or eliminating microorganisms are implicated for contamination prevention during food processing and storage. In spite of this, food-borne pathogen bacteria can be found in ready-to eat foods and represent a hazard for consumers' health.[1–3] Sampling and laboratory methods are routine in permanent food safety assay and detection of food pathogens. *L. monocytogenes* and *Escherichia coli* are the main bacterial pathogens listed in microbial criteria of foods, and their control requires quantitative and qualitative detection.[4] The time of testing of laboratory protocols for detection and identification of food-borne bacteria is of great importance in the assay of short-term shelf-life foods. There is a need for very fast determination of food safety levels, to establish the term of "use before" in such a group of products. Routinely used microbiological methods based on culturing techniques are time consuming, and spend at least 24 to 72 hours. Immunological and molecular-biological methods are faster but are still expensive.

Several researchers have shown the possibility of spectroscopy in identification, classification and differentiation of bacterial species.[5–9] Differences in biochemical properties and composition (fatty acids, carbohydrates etc.) in bacterial species allow the use of chemometrics in model development, based on infrared or FT-near infrared spectroscopy data from bacteria.[10,11]

Fast, accurate and cheap detection of bacteria in different media is also currently an important research area in the medicine, food and agricultural sciences. The aim of our investigation was detection of *L. monocytogenes* and *E coli* bacteria on the surface of artificially contaminated sausages and cheese by NIR spectroscopy, in combination with multivariate chemometrics techniques.

Materials and methods

Vacuum-packed sausages and cheese were opened under aseptic laboratory conditions, cut into pieces with 4 mm thickness, and put into Petri dishes with a diameter of 5 cm. A total of 42

samples were prepared from each product. The product surface of 28 samples was contaminated with *L . monocytogenes* (n = 14) and *E. coli* (n = 14). Contamination was achieved by the spread of 0.1 ml from broth dilutions containing 10^3 CFU/ml of overnight bacterial broth culture. Control samples (n = 14) were prepared by adding the same aliquot of sterile broth. All samples were vacuum packed and stored in a refrigerator at approx. 10ºC for 12 days. Control samples, and samples contaminated with bacteria were scanned by NIR in triplicate at days 0, 2, 4, 6, 8, 10 and 12. After spectral measurement, microbiological analysis of each sample was performed.

NIR measurements were performed by an NIRSystems Model 5000 in the wavelength region 1100–2500 nm, using the reflection mode. A commercial program Pirouette, Version 2.0 (Infometrics, Inc., Woodinville, WA, USA) was used for performing spectral data processing. Class variables were assigned for each sample as follows: control samples (class Control), samples infected with *L. monocytogenes* (class *L.mono*) and samples infected with *E. coli* (class *E.coli*). Soft Independent Modeling of Class Analogy (SIMCA) was implemented to create models of the respective classes, based on NIR spectra of contaminated and control samples. PLS regression was used for quantitative analysis.

Results and discussion

Concentration of bacteria in contaminated sausage samples increased during the storage from 1.6×10^3 to 1.7×10^4 CFU/g for *E. coli* and from 8.0×10^3 to 3.1×10^7 CFU/g for *L. monocytogenes.* Respective values for cheese samples were from 1.7×10^3 to 1.7×10^4 CFU/g for *E. coli* and from 5.5×10^2 to 1.8×10^4 for *L. monocytogenes* bacteria. No bacterial contamination was established in the control samples.

SIMCA models for sausage and cheese samples are presented in figures 1 and 2, respectively. There are clear differences and distances between all 3 groups of strain contaminated and non-contaminated samples.

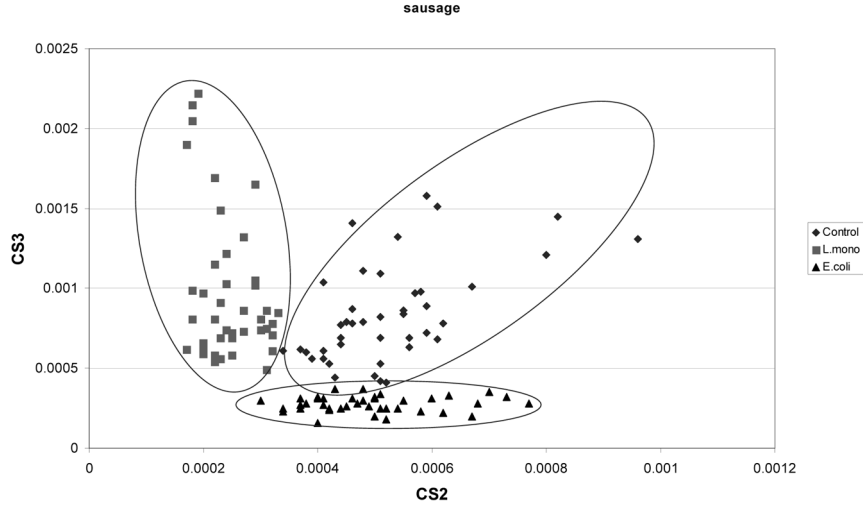

Figure 1. SIMCA classification of sausage samples.

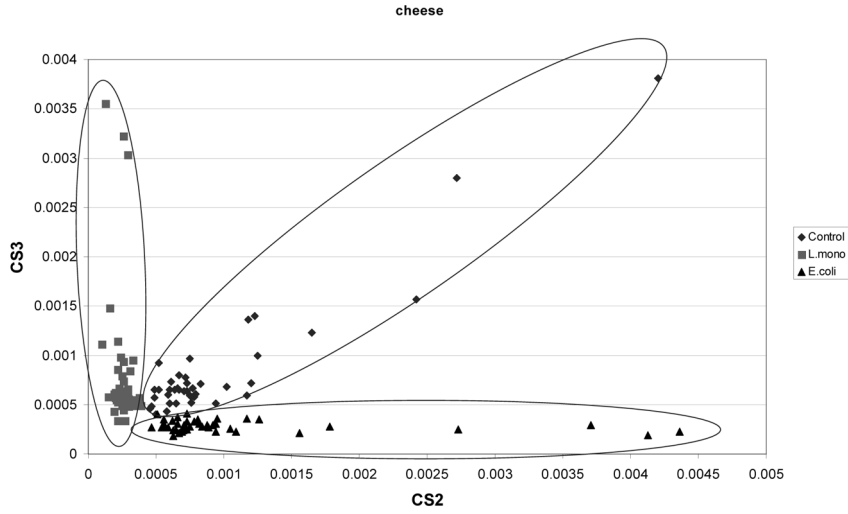

Figure 2. SIMCA classification of cheese samples.

Our results showed that SIMCA models correctly classified all sausage and cheese samples from class *L.monocytogenes* (Table 1).

Despite the low level of contamination of cheese and sausage samples with *E. coli* bacteria, SIMCA models correctly classified 90% of sausage samples and 95% of the cheese samples. No control samples from sausage were classified as contaminated, and only one cheese sample was incorrectly classified.

SIMCA models are based on principal component analysis of spectra of samples, belonging to the respective classes. The parameter "Modeling Power" showed variables that have importance for description of spectral information presented in certain classes of samples. In our case differences in "Modeling Power" would show differences in spectra of samples of the three tested classes. Differences in modeling power plots were observed as between class Control and class *L.mono* or class *E.coli*, as well as between class *L.mono* and class *E.coli* for both tested products. The plot of "Modeling Power" for cheese samples was presented at Figure 3.

The most significant differences between classes were observed in region 1164-1380 nm, around 1440 nm, 1530 nm, 1670 nm, 1820 nm and in region of 2390-2420 nm for both cheese and

Table 1. SIMCA classification of sausage and cheese samples.

	Sausage samples			Cheese samples		
	Class Control	Class *L. mono*	Class *E. coli*	Class Control	Class *L. mono*	Class *E. coli*
Class Control	42			41		1
Class *L. mono*		42			42	
Class *E. coli*	4		38	2		40

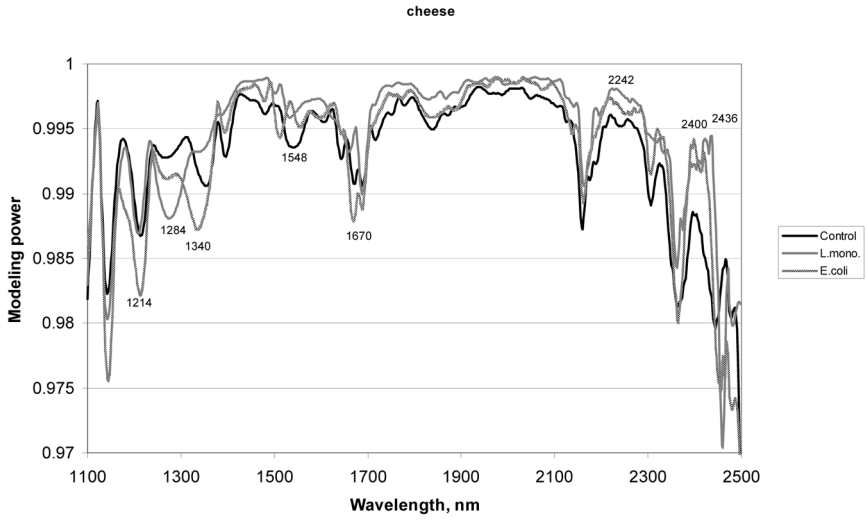

Figure 3. Plot of parameter "Modeling Power" for cheese samples—class *L. monocytogenes,* class *E. coli and* class Control.

sausage samples. Additional significant differences were found between 2142 nm and 2170 nm for sausage samples and around 2240 nm and 2316 nm for cheese samples.

Results of quantitative determination showed high correlations between NIR spectra and bacterial contamination of tested samples. Statistical parameters of PLS equations for determination of *E. coli* concentration were: in sausage samples $SECV = 0.21$ logCFU/g and $R = 0.91$; in cheese samples $SECV = 0.20$ logCFU/g and $R = 0.97$. Respective values for determination of *L. monocytogenes* concentration were: in sausage samples $SECV = 0.78$ logCFU/g and $R = 0.90$; in cheese samples $SECV = 0.30$ logCFU/g and $R = 0.93$.

Conclusion

In conclusion, our initial results showed that near infrared spectroscopy in combination with multivariate chemometrics data analysis offers an alternative approach to conventional methods, with large potentials for a rapid and reliable identification in the area of microbiology and food safety. It was possible to establish SIMCA models for detection of food samples, contaminated with *L. monocytogenes* or *E. coli* bacteria, and PLS algorithms for quantitative estimation of bacterial contamination in these foods.

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References

1. N. M. Sachindra, P. Z. Sakhare, K. P. Yashoda and D. Narasimha Rao, *Food Contr.* **16,** 1 (2007).
2. S. Temelli, S. Anar, M. K. C. Sen, D. Beyaz, *J. Fac. Vet. Med*. **24,** 1 (2005).
3. C. M. Lin, K. Takeuchi, L. Zhang, C. B. Dohm, J. D. Meyer, P. A. Hall, M. P. Doyle, *J. Food Prot*., **69,** 1 (2006).
4. Anonymous, *Off.l J. Europ. Union* **L 322,** 12 (2006).
5. D. Nauman, D. Helm and H. Labischinski,. *Nature* **351,** 8182 (1991).
6. P. Whittaker, Mossoba M.N., Al-Khaldi S., Fry F. S., Dunkel V.C., Tall B.D., and Yurawecz M.P., *J. Microbiol. Methods* **55,** 3 (2003).
7. M. Lin, M. Al-Holy, M. Mousavi-Hesary, H. Al-Qadiri, A.G. Cavinato and B.A. Rasco, *Lett. Appl. Microbiol*. **39,** 148 (2004).
8. A.Oust, T. Møretrø, K. Naterstad, G. Sockalingum, I. Adt, M. Manfait, and A. Kohler, *Appl. Environ. Microbiol*. **72,** 1 (2006).
9. D. Alexandrakis, G. Downey and A.G.M. Scannell, *J. Agric. Food Chem*. **56,** 10 (2008).
10. C. Rebuffo, J. Schmitt, M. Wenning, F. von Stetten, S. Scherer, *Appl. Environ. Microbiol.* **72,** 994 (2006).
11. C. Rebuffo, J. Schmitt, and S. Scherer, *Appl. Environ. Microbiol.* **73,** 3 (2007).