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# SOFTWARE PACKAGE FQPM - FOOD QUALITY PREDICTION MODELS USING SPECTRAL DATA

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## Software FQPM - predikční modely parametrů kvality potravin s využitím spektrálních dat

## Abstract

Software package FQPM (Food Quality Prediction Models using Spectral Data) consists from:
- framework and methods for predicting food quality & safety parameters using different spectral data,
- examples of usage of designed methods for different issues in food control and quality,
- datasets gathered during resolving of project VG20102015023 with usage of hardware equipment developed under this project,
- robust prediction models for selected food quality issues, i.e.:
  - Prediction model for basic beef components and quality parameters (solids, protein, fat, N-based substances, water binding capacity, pigment content, pH, light remission, ash content, cutting power) using resistance spectra of beef and PLS calibration against reference methods for all of components.
  - Model and usage of average FTIR milk spectra deducted from large (~78 ths spectral database of milk samples used for animal recording) spectral database and its usage for detection of abnormal (diluted, manipulated, etc.) milk samples using FTIR spectroscopy using database confidence intervals
  - Dataset and prediction model for predicting of meet aging under the standard conditions and content of biogenic amines (BA) as a marker of meet safety using changes of its NIR UV/non-UV spectra.
  - Prediction models for SCC (Somatic Cells Count) in raw milk using resistance and phase shift spectral data.
  - Prediction models for different type (raw, UHT) milk and its different stocking conditions predicting robustly time of storage of milk with usage of resistance and phase shift data.

Software package allows users to use developed models in their own hardware equipment as the whole package is developed in The Unscrambler X v10.3 (CAMO software, 2013) software which is strongly used in industrial spectral R&D and by plenty of manufacturers of spectral devices in food industry. In case of users want to use another hardware platforms then have been using during our development, it even illustrates and explains how different principles of spectral analysis can be applied in food safety and quality and allows user to adopt different data analysis and processing methods as well as prediction methods. In the meaning of basic research, software package shows clearly that methods used to gather data and methods of data processing and modelling can be used for the purposes in food S&Q listed above.

**Keywords:** Food Safety & Quality prediction, bioimpedance, spectral data

## Abstrakt

Software FQPM (Food Quality Prediction Models using Spectral Data) sestává z:
- rámcové definice postupů a metod pro predikci parametrů potravinové kvality a bezpečnosti s využitím různých spektrálních dat,
- příkladů použití navržených metod pro různé úlohy v kontrole potravinové kvality a bezpečnosti,
- datových sad spektrálních dat získaných během řešení projektu VG20102015023 za pomoci hardwarových řešení vyvinutých v rámci projektu,
- robustní predikční modely pro vybrané úlohy potravinové kvality, t.j.:
  - Predikční model pro složení hovězího masa a jeho kvalitativní parametry (bílkoviny, sušina, tuk, dusíkaté látky, vaznost vody, obsah pigmentů, pH, remisi světla, obsah popelovin, střižnou sílu) s využitím spektra rezistance (impedance) hovězího masa a PLS kalibrace na referenční metody pro všechny popsané parametry.
  - Model a využití průměrného FTIR spektra syrového mléka odvozený z rozsáhlé (cca 78 tisíc vzorků) spektrální databáze a jeho využití pro detekci abnormálních (ředěných, manipulovaných, atd.) vzorků mléka pomocí FTIR spektroskopie a intervalů spolehlivosti spektrální databáze.
  - Datový set a predikční model pro predikci doby skladování masa ve standardních podmínkách a obsah biogenních aminů jako ukazatele bezpečnosti masa pomocí změn NIR UV/non-UV spektra vzorku masa.
  - Predikční modely pro obsah somatických buněk v syrovém mléce pomocí spektrálních dat impedančních (rezistence, posun fáze) spektrálních dat.
  - Predikční modely pro různé typy (syrové, UHT) mléka a jejich skladovací podmínky predikující dobu skladování mléka s využitím impedančních spekter.

Softwarový balík umožňuje uživateli využít vytvořené modely s vlastním hardware, neboť je vytvořen s využitím SW The Unscrambler X v10.3 CAMO software, 2013),

jehož predikční engine využívá, a který je plošně používán ve výzkumném a vývojovém prostředí, stejně jako v průmyslovém prostředí výrobci spektrálních analyzátorů pro potravinářský průmysl. V případě, že uživatelé chtějí používat vlastní HW řešení, software ilustruje způsob použití spektrálních dat pro dané úlohy a ukazuje, jaké metody předpřípravy dat, jejich analýzy a predikčních modelů pro dané úlohy využít. Z hlediska výzkumu v oboru software ukazuje, že použité metody pro získání spektrálních dat, metody pro jejich zpracování a analýzu a metody pro modelování mohou být využity pro úlohy potravinové kvality a bezpečnosti uvedené výše.

**Klíčová slova:** Predikce parametrů potravinové kvality a bezpečnosti, bioimpedance, spektrální data

## Introduction

At the beginning, we want to introduce briefly spectral methods used for dataset included in package creation process.

Infrared (IR) spectroscopy is based on the principle that the chemical bonds in organic molecules absorb or emit infrared light when their vibrational state changes. Large changes in vibrational state are observed in the near IR part of the spectrum (NIR), and sometimes up to the visible, while primary vibrations are produced in the mid IR region. Interrogation of NIR datasets by increasingly powerful and sophisticated chemometric techniques continues to improve calibration robustness and accuracy (Damez J. L., 2013).

Several IR spectroscopy techniques exist. The main differences are the bandwidth and the optically measured parameters (transmission, reflection, diffusion, scattering, etc.). Treatment also differs according to the use or not of Fourier Transformation (FT-IR spectroscopy) before exploiting the spectra (Damez J. L., 2013).

NIR (FTIR) is implemented in food analysis. For example new method using VIS and NIR light transmission was developed to evaluate the quality of beef meat based on marbling detection (Ziadi, 2012). Fat content in fish is measured by NIR transmittance spectroscopy (Nortvedt, 1998).

Impedance, also called bioimpedance, is widely used technique to characterize the properties of biological tissues (Damez, 2007). In general it is physical quantity describing the apparent resistance of the measured material and depends on the frequency of alternating current (AC) used. Impedance is expressed by a complex number and therefore consists of 2 parts: resistive (real) and capacitive (imaginary). Impedance Z is defined as a ratio of voltage and current quantities, which relating the AC could be phase shifted (Damez J. L., 2013).

The most elementary and commonly used measurement apparatus are two electrodes to induce a current flow (I) and a voltage (V) between these two electrodes, making it possible to deduce an electrical impedance by applying Ohm's law, V = ZI. The impedance (Z) is a complex function of alternating current frequency f, e.g. $Z = Z_{real} + iZ_{imag}$, where $Z_{real}$ is the real part (resistive), $Z_{imag}$ the imaginary part (capacitive) and $i = (-1)^{1/2}$ (Damez J. L., 2013).

Relating the biological tissues high frequency spectra are used to measure the impedance, because only this way AC passes through the cell membranes and induce both - resistive and capacitive part of impedance (Damez, 2007).

Commonly impedance spectroscopy is used for measurement of meat tenderness (Lepetit, 2002), fat content (Swantek, 1992), salt content (Chevalier, 2006) and also the fish freshness (Pérez-Estevea, 2013).

NIR and Visible light spectrum (VIS) hyperspectral analysis is widely used as non-invasive method for a meat spoilage detection. As an emerging and innovative tool, originally developed for remote sensing applications, hyperspectral imaging technique (HSI) combines the traditional spectroscopy and computer vision technology into one system and provides a three-dimensional hypercube (x, y, $\chi$) including spectral ($\chi$) and spatial (x and y) information simultaneously. The obtained hyperspectral images are composed of hundreds of contiguous wavebands for each spatial position of a target studied. Accordingly, the spatial-feature enables characterisation of complex heterogeneous samples and image texture, while the spectral-feature allows for the identification of internal chemical information (Jun-Hu Cheng, 2015).

## Material & Methods

### *Prediction model for basic beef components and quality parameters using resistance spectra of beef and PLS calibration.*

Eleven samples of beef tenderloin have been analysed using reference laboratory methods for following parameters: solids, fat, protein, N-based substances, pigment and ash content. Also, water binding capacity, pH, light remission and cutting power have been measured as qualitative parameters of beef quality.

Impedance spectra (resistance) have been scanned in a range of 1 kHz - 100 KHz with a step of 500 Hz using the pair of needle electrodes and the prototype spectrometer developed during project.

Impedance spectra were used to build prediction models for all of parameter using PLS algorithm (mean centered spectra, NIPALS, 11 components, 7 factors) and cross-validated.

### *Model and usage of average FTIR milk spectra deducted from large spectral database and its usage for detection of abnormal (diluted, manipulated, etc.) milk samples using FTIR spectroscopy*

Large database of different milk samples from milking cows (78 215 samples) has been used to calculate an average FTIR spectra of raw milk with different quality but safe. Spectra from Bentley Instruments FTS have been used as it represents an instrument commonly used for raw milk sample analysis and it is robustly and developer-friendly manufactured. FTIR spectra have been gathered in

897 commonly used data points from 649 up to 3991 $cm^{-1}$. This is again because of this setup of spectrometer is used in practice.

Then, different confidence limits (99, 95, 90%) have been calculated across all of spectra data points to establish limits for "normal" milk sample. These results have been validated against milk samples and reference QSE standards.

### *Dataset and prediction model for predicting of meet aging under the standard conditions and content of biogenic amines as a marker of meet safety using changes of its NIR UV/non-UV spectra.*

Raw meat sample (300 g slice of the pork shoulder; bought in a common supermarket) was inoculated by wild type bacteria mixture isolated from a spoiled meat in Tryptonic-Soy broth by immersing whole slice into 800 ml of bacteria suspension for 10 minutes. Meat sample was dried and 15 g sample for biogenic amines content assay was cut immediately. Then both optical and impedance spectra were recorded by the 20 minutes interval for 5 days and 15 g slice was cut for biogenic amines content assay. The net results were used as training data for a prediction model calibration.

VIS/NIR spectra of beef was captured by using AvaSpec-ULS2048 portable spectrometer and SL1 Stellar net Tungsten Halogen lamp from 350 nm to 1100 nm. Biogenic amines content has been analysed by the reverse phase HPLC according the article by Smělá (2003). Impedance spectra were scanned in a range of 200 Hz - 100 KHz using the pair of needle electrodes and the prototype spectrometer developed during project.

50 gathered NIR spectra for pork sample have been calibrated using mean centered PLS algorithm with wide kernel modification and then cross-validated in Unscramler X for 3 factors - time of aging (in days) and for two of different methods for BA content.

### *Prediction models for SCC (Somatic Cell Count) in raw milk using resistance and phase shift spectral data.*

38 different samples with 5 different levels of somatic cell content (counted by direct microscopy) have been analyzed using impedance spectrometer. Both, resistance and impedance spectra from 1 kHz - 100 kHz with 500 Hz step (raw, normalised) datasets in combination with PLS (NIPALS), have been used to investigate which is the most robust and precise method and dataset to build calibration for SCC using impedance spectra. All of calibrations have been cross-validated then.

### *Prediction models for different type (raw, UHT) milk and its different stocking conditions predicting robustly time of storage of milk with usage of resistance and phase shift data.*

Three models for prediction of time of stocking of milk have been built using impedance spectra (1 kHz - 100 kHz, 500 Hz step). Time in minutes of stocking has been used as calibration parameter. Samples have been stored in different conditions and analysed in each time of analysis using flow system so, every time new stroke of milk sample has been measured.

UHT milk with simulated defected packing (UHT milk poured into non-sterile vial) was used for the first model. Sample has been analyzed twice for each time of analysis (0 - 400 minutes). Impedance spectra has been used to build PLS calibration illustrates milk sample spectra performance in time and also to show how precise this parameter could be predicted.

Principal component regression of impedance spectra has been used for raw milk sample degradation monitoring description and prediction. Milk sample has been warmed up and put into room temperature (22 0C) and then analysed in 0 - 60 minutes with step of 2 minutes.

The same experiment has been done using UHT milk sample then and the best prediction model has been calculated as well.

## Results & Discussion

Goal of this article is to describe software package FPQM. In this article, we want to present even capability of prediction models created to predict factors used in each experiment as the main goal of it. So, we do not want to discuss deeply results obtained for each parameter and we would like to refer to FPQM package directly to see how raw data, their distributions etc. have looked like. We illustrate model performance also only by two basic measures - R-square coefficient and RMSE (root mean square error of prediction) for cross validated models. This is showing effectively and in the frame of article, what is possible to achieve in all of very different subjects described here. It is not a goal of article to discuss deeply results obtained in each experiment or even to formulate and prove hypothesis why results are like they are in each topic.

### *Prediction model for basic beef components and quality parameters using resistance spectra of beef and PLS calibration.*

Following results have been obtained for models predicting beef components content and quality parameters:

***Table 1.*** *R-Square coefficients and RMSE for PLS calibrations for beef components content and quality parameters.*

| Parameter | R-Square | RMSE |
|---|---|---|
| Solids | 0.614 | 1.216 |
| Fat | 0.653 | 1.182 |
| Protein | 0.661 | 0.313 |
| N-based subst. | 0.636 | 0.053 |
| Pigment | 0.739 | 0.364 |
| Ash | 0.445 | 0.031 |
| Water binding capacity | 0.856 | 1.898 |
| pH | 0.508 | 0.201 |
| Light Remission | 0.694 | 0.844 |
| Cutting Power | 0.579 | 2.41 |

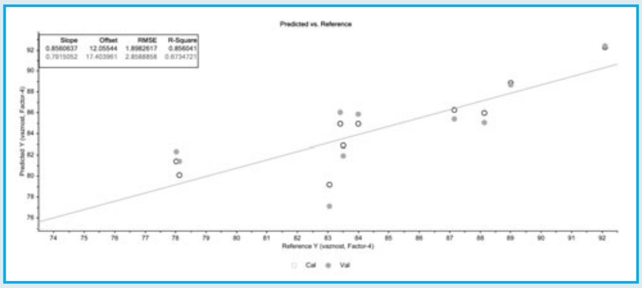



*Figure 1.* Performance of water binding capacity prediction model.

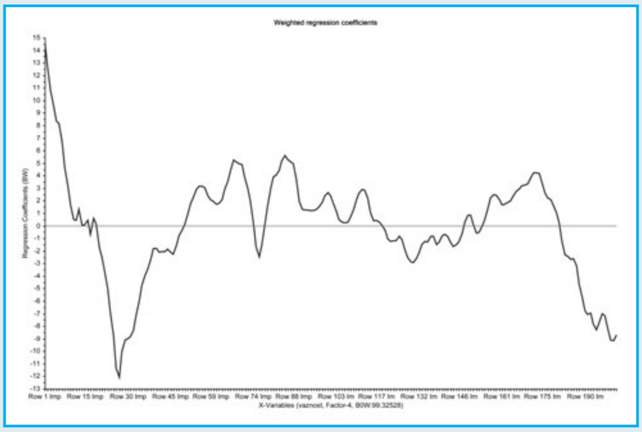



*Figure 2.* Regression coefficients for impedance spectra based model for water binding capacity.

### *Model and usage of average FTIR milk spectra deducted from large spectral database and its usage for detection of abnormal (diluted, manipulated, etc.) milk samples using FTIR spectroscopy*

Figure 3 bellow shows an average milk FTIR spectra deducted from database of spectral data, lower and upper 90% confidence intervals and projection of milk samples and one sample of QSE (deeply frozen) sample. It is obvious that there are differences in noise areas as well as 90% confidence intervals created from as large dataset as we used are to robust here to just have been used as discrimination parameter for manipulated/non-standard milk samples. It is also obvious the effect of deep freezing on milk spectra in QSE standard. However, any other metrics of similarity to average spectra can be calculated and used.

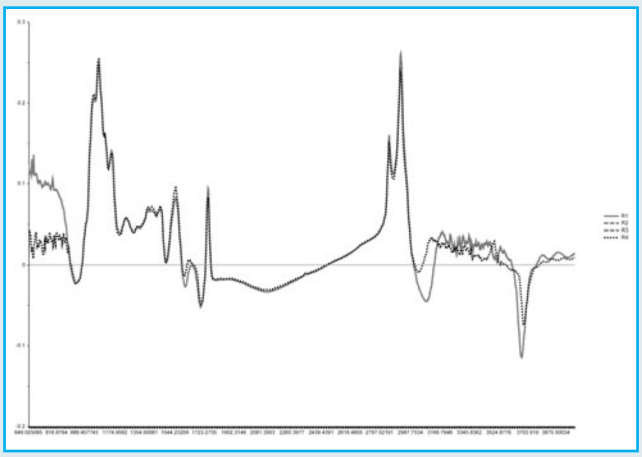


*Figure 3.* Average FTIR raw milk spectra and sample projections (Legend: R1-R3 - average spectra and confidence intervals; R4 - spectra of manipulated sample).

### *Dataset and prediction model for predicting of meet aging under the standard conditions and content of biogenic amines as a marker of meet safety using changes of its NIR UV/non-UV spectra.*

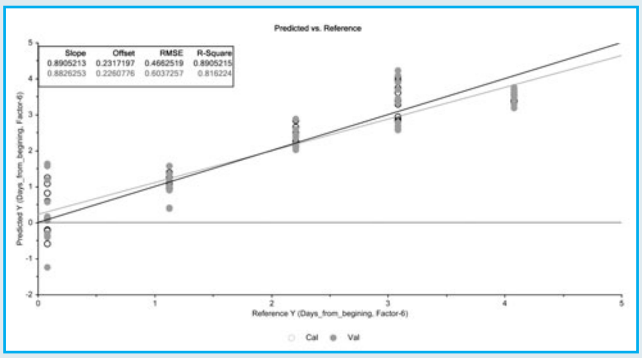
Performance of meat aging prediction model using NIR spectra is illustrated on figure bellow.

As we supposed, there is the biggest variation in prediction in the beginning of experiment as this variation is caused mainly by different samples content than their changes in time. Anyway, including this, the model is able to predict day of aging with acceptable parameters (R-Square=0.816; RMSE=0.466) as its validation shows.

Two methods of biogenic amines have been used to develop calibration models based on NIR spectra as reference data. The model for the first method shows R-Square coefficient equals to 0.788 and RMSE equals to 13.553. The model for the second method then shows R-Square coefficient equals to 0.810 and RMSE equals to 11.080 after its validation.

### *Prediction models for SCC (Somatic Cell Count) in raw milk using resistance and phase shift spectral data.*

Five reference samples have been analysed in this experiment with different levels of somatic cell content (from



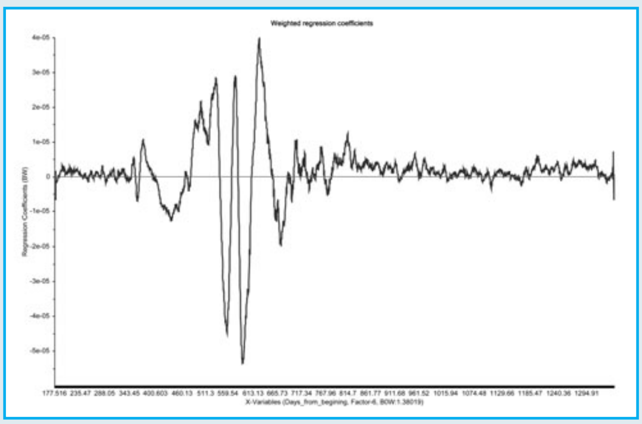




*Figure 4 and 5.* Performance of meat aging prediction model using NIR spectra and NIR spectra correlation coefficients for model.

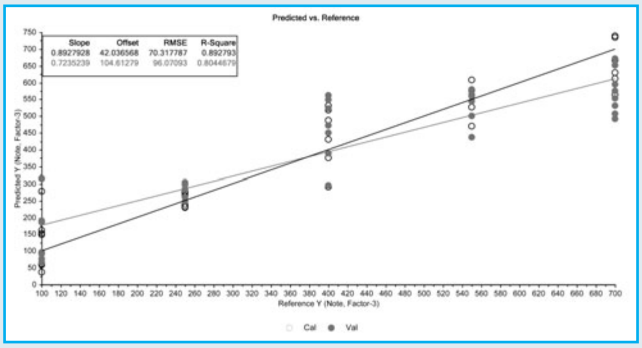



***Figure 6.** Performance of prediction model based on normalized phase shift data for SCC.*

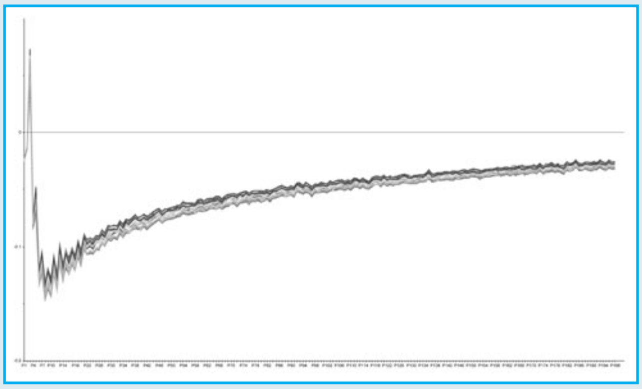


***Figure 7.** Normalized phase shift spectra of milk samples.*

100 up to 700 ths. of SCC per ml). Then, different data processing methods and the both of spectra measured (resistance and phase shift spectra) have been used to build up robust prediction model. The best results in this experiment have been obtained for model uses normalized phase shift spectra as predictor - see figure 6.

### *Prediction models for different type (raw, UHT) milk and its different stocking conditions predicting robustly time of storage of milk with usage of resistance and phase shift data.*

Figures 8, 9 and 10 illustrate how time of stocking of UHT (figures 8 and 10) and raw milk (figure 9) can be

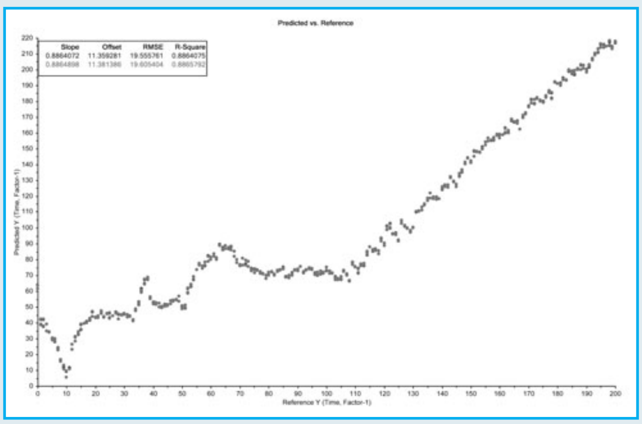



***Figure 8.** Predicted changes in time of stocking of UHT milk put into non-sterile vial (defected packing simulation) projected using PLS calibration of resistance spectra into the time space.*

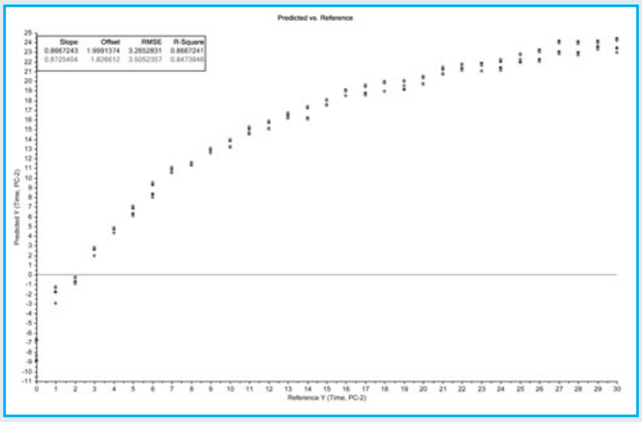



***Figure 9.** Predicted changes in time of stocking of raw milk in room temperature conditions projected using PLS calibration of resistance spectra into the time space.*

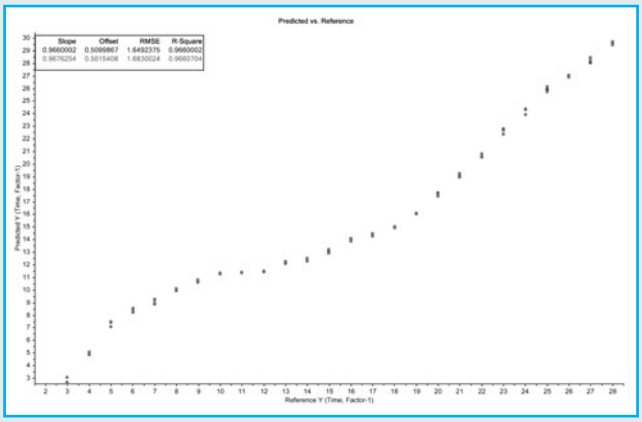



***Figure 10.** Predicted changes in time of stocking of UHT milk in room temperature conditions projected using PLS calibration of resistance spectra into the time space.*

projected using PLS calibration based on resistance spectral data and how precisely the time of stocking can be predicted.

## Conclusion

Software package FPQM presented in this article shows how to use different type of spectral data for different issues in food quality. It was created with usage of experimental results with regard to its use in real industrial applications. Results obtained during its creation shows that even models created for the purpose of package are ready to use in actual industrial applications (SCC prediction model, average spectra application) and future applications of devices developed during the project no. VG20102015023 (aging of meat, prediction of time of stocking of food resources etc.).

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**Note:**

The FPQM software package is contractually licensed to Bentley Hungary Kft. and Bentley Instruments S.A.R.L to be used in company R&D and actual and future instruments for milk analysis.

Methods and models presented in this article are results of R&D done under the project no. VG20102015023 and they represent different new ways how to use commonly used and developed spectral methods in practice in food safety and quality issues. They also represent the practical way of usage of instruments developed under the project.

Two users of software are licensed up to today - who are manufacturers of milk analyzers. We have estimated value of financial benefit for them as 16.200.000 CZK as a costs saved by R&D investments which should has been invested if FPQM package was not created and as income of instruments implementing models and methods to be sold with usage of competitive advantages given by implementation of package into the instruments.

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# VLIV KULTIVAČNÍCH PODMÍNEK NA TVORBU NISINU PŘI FERMENTACI SYROVÁTKY KMENY LAKTOKOKŮ

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## Influence of cultivation conditions on nisin formation during fermentation of whey by *Lactoccoccus* strains

## Abstrakt

Pro sledování tvorby nisinu u 3 vybraných kmenů *Lactococcus lactis* byla zvolena agarová difuzní metoda na BHI agaru s indikátorovým kmenem *Micrococcus luteus* a optimalizovány podmínky metody. Testované kmeny byly pro matečnou kulturu kultivovány na M17 agaru, v mléce, v sladké syrovátce a v BHI bujonu, potom očkovány do mléka a sladké syrovátky. Výrazně nejrychlejší koagulace byla zaznamenána u kmene CCDM 731 - po 16 hod. Kmen byl dále kultivován v sladké syrovátce stacionárně i s protřepáváním po dobu 0 až 42 hod. Aerace substrátu neměla vliv na tvorbu nisinu, maximální koncentrace nisinu byla zjištěna již po 4 - 8 hodinách. Při testování různých suplementů do syrovátky pro zvýšení tvorby nisinu se jako nejlepší ukázalo použití 0,1 % Tweenu 80.

**Klíčová slova:** nisin, laktokoky, syrovátka, nisin-produkční kmeny

## Abstract

The agar diffusion method on the BHI agar with use of the strain indicator *Micrococcus luteus* was selected for monitoring of nisin produce by 3 strains of *Lactococcus lactis* and the method conditions were optimized. Tested strains were cultured for the mother culture on M17 agar and in BHI broth, milk and sweet whey, then inoculated to milk and sweet whey. The strain CCDM 731 after 16 hours appeared significantly the fastest coagulation. This strain was further cultured in sweet whey stationary or with shaking for 0 - 42 hours. The aeration of the substrate did not affect the formation of nisin, the maximum concentration was detected after 4 - 8 hours. Testing of various suplements in the whey to increase nisin produce proved the best result use of 0,1 % Tween 80.

**Key words:** nisin, lactococci, whey, nisin producing strains

## Úvod

Bakteriocin nisin je přírodní látka s širokou antimikrobiální aktivitou vůči technologicky nežádoucí, ale i patogenní mikroflóře potravin. Jedná se o peptid, který je produkován bakterií mléčného kvašení *Lactococcus lactis.* Světovou zdravotnickou organizací (WHO) je schválen jako potravinářské konzervační aditivum. Nisin se používá zejména jako ochrana v tepelně ošetřených potravinách a v potravinách s nízkým pH. Výhodou ošetřených potravin je prodloužená doba minimální trvanlivosti a úspora výdajů ve výrobě - snížením teploty a zkrácením času tepelného ošetření. Významná je schopnost zajištění bezpečnosti potravin působením proti G+ bakteriím, jejich sporám a zejména proti významným patogenům *Listeria monocytogenes, Bacillus cereus* a *Clostridium botulinum.* O bakteriocin nisin je mimo potravinářský průmysl zájem též v medicíně a v léčbě mastitid skotu (Lertchaowayuth a spol., 2013; Delves - Brouhton a spol., 1996).

Přestože je nisin již komerčně vyráběn, celá řada výzkumných prací se zabývá problematikou, jak nisin vyrobit levně a efektivně. Syrovátka je vhodným levným médiem. Dosud tato surovina není dostatečně využívána.