Innovative engineering methods for quality evaluation and food safety

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Abstract: The improvement of quality of life and human activity has many directions. One of them is providing high-quality and safe food. Advancements in sensor technologies, data mining and processing algorithms have provided technical capabilities for development of innovative engineering methods that guarantee certainty regarding the quality control of food and public health. The potential of Near Infrared Spectral Analysis and Aquaphotomics as non-destructive and rapid methods for monitoring food quality through observation of water absorbance bands is presented.

Key words: near infrared spectroscopy, aquaphotomics, multivariate data analysis, food safety and control

1. Introduction

A high level of public health is one of the main tasks for improving quality of human's life. The public interest relating to the quality and production methods of food and food control increases significantly in recent decades. Strategies of many countries are aimed towards determining regulatory standards against food fraud, low quality, bacterial control, etc. Their rules are connected to hygienic production, storage and transportation. Meanwhile, recent researches show a lot of cases of mass or sporadic poisoning with ready-to--consumption products dangerous to their users (Kitamoto et al., 2009; Rouger et al., 2017; Schmid et al., 2007; Zhang et al., 2012). It is also a fact that traditional techniques for food control are precise and objective but also too laborious, lengthy, expensive and destructive. The necessity of developing modern engineering methods that can be used directly in farms, on food production lines, is growing. Quick responses are particularly important to industry, where an answer is needed within minutes. That would allow to take appropriate corrective measures in a timely manner, directed towards protecting people's health (Cheng et al., 2013; Cozzolino and Murray, 2012; Rukchon et al., 2014; Teixeira dos Santos et al., 2013; Weeranantanaphan et al., 2011).

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Near-infrared (NIR) spectroscopy opens a new area in biotechnology, food science, and engineering by exploring and describing biological systems through a rapid non-destructive monitoring of their interaction with NIR light. This method is based on the absorption of C-H, N-H, O-H, and S-H molecular bonds that are present in most organic raw materials and products and uses the range from 780 to 2500 nm of the electromagnetic spectrum (Sandorfy et al., 2007).

Near-infrared spectroscopy is a very flexible technology and has many advantages. This technique allows samples in different physical phases: solids, liquids, pasty and gases, to be measured directly without any pre-treatment (Feng and Sun, 2013; Jia et al., 2017; Huang et al., 2014). Water has less absorbance in the NIR region, compared to the absorbance in the mid-infrared one. Thus, aqueous solutions, food or biological samples, which usually contain a large amount of water, can be measured directly without complicated sample preparation procedures and waste materials such as toxic solvents. Samples could be measured through glass (glass cuvettes, glass vials or sample cups with glass windows) or even through transparent packaging (Veleva-Doneva, 2012; Zhang et al., 2012). Remote measurements using quartz fiber optics are possible and could be used for online process monitoring. Because of the non-destructive nature of the analysis, the samples can be used for further tests (Cheng et al., 2013; Feng and Sun, 2013; Veleva-Doneva et al., 2010). An additional advantage of NIR spectroscopy is that spectra often contains information about physical properties such as particle size, viscosity, density, temperature, pH, dry matter, fat, colour, etc. (Atanassova et al., 2017; Balage et al., 2015; Collell C. et al., 2012).

Successful application of NIR spectroscopy depends on the correct choice of spectral instrument. The choice of the instrument depends on analyzed products, their chemical composition and structure, required analyte sensitivity and selectivity, reliability, ease-of-use, and implementation needs. Modular-configurable instruments with a range of light sources, fibers, accessories for different measurement mode, allow easy measurement of specific products. Fiber optic probes are very good solution for sampling in-vivo, analyzing large samples, monitoring real-time reactions, and any other application where it is difficult to bring the sample to the spectrometer. The flexibility and user-friendliness has made them one of the most widespread tools in modern spectroscopy. Fiber-optic probes have the widest range of applications in laboratory and online analysis of different food samples such as milk, dairy products, meat, meat products, fish, fish products, fruit, beverages, etc. (Horvath et al., 2008; Tito et al., 2012; Trocino et al., 2012; Niu et al., 2014; Reis and Rosenvold, 2014). Development in optics and electronics lead to miniaturization of NIR instruments. Recently offered small size and low cost portable NIR spectrometers have several advantages for non-destructive, online, or in-situ analysis in agro-food industry (Teixeira dos Santos et al., 2013).

In the last fifteen years NIR spectroscopy has been used in combination with a new tool caled Aquaphotomics. This approach has been developed by prof. Roumiana Tsenkova from Kobe University, Japan and can be used to find water hydrogen bonds in different aqueous systems under various perturbations. The goal changes in water to be utilized to obtain more information for understanding changes in observed biological system. Twelve characteristic wavelengths known as 'WAter MAtrix CoordinateS' (WAMACS) have been identified in the area of the first overtone of the water NIR spectra, where despite the type of perturbation

the observed systems showed predictable spectral variations. Changes of water absorbance pattern can be visualized as a star chart named 'Aquagram' (Tsenkova, 2009). Aquaphotomics has been applied in different areas as water characterization, food quality control, early diagnostics in medicine, etc. (Matija and Tsenkova, 2011; Munćan, 2011; Lu et al., 2016; Veleva-Doneva, 2017).

The use of Aquaphotomics based on spectral information for analytical purposes relies on the multivariate data analysis. Nowadays this is of even more importance because of the increasing power of computers and the development of NIR spectrophotometres that allow thousands of spectral data points to be obtained. Aquaphotomics is the analytical technique which the most applies chemometrics as a tool for extracting relevant information from analytical data (Atanassova et al., 2017; Feng and Sun, 2013; Kumar et al., 2014). NIR spectroscopy has been successfully used by Huang et al., 2016 for intramuscular fat investigation in fresh, frozen and frozen-thawed pork meat. They obtain the best results in first derivative of raw spectra and application of Gabor filtered mean spectra. Wu et al., 2016 have applied Partial least squares regression (PLSR) to design a prediction model for pork meat exposed to freeze-thawed processes with correlation coefficient of prediction 0.81 and root mean square error (RMSE) 0.33. Similar evaluation procedure has been applied by Xiong et al., 2015 with regression coefficients in prediction (Rp) 0.944 and root mean squared errors estimated by prediction (RMSEP) 0.081. Li et al., 2016 have studied meat pork quality to differentiate normal from pale, soft and exudative meat (PSE). Niu et al., 2014 have applied NIR spectra to make a classification and prediction models for identification donkey, beef, pork and mutton meat by SIMCA method. Accuracy of 100% for calibration and 98% for prediction has been achieved. Spectral instrument has been used by Collell et al., 2012 in prediction of water activity and moisture content in fermented dry sausages. Atanasova, 2015 has investigated NIRS in combination with aquaphotomics as a tool for monitoring the changes during ripening of Bulgarian yellow cheese from cow milk. She found significant changes in aquagram patterns depending on changes in titratable acidity and protein fraction in cheese during ripening. Cattaneo et al., 2016 have studied the differences in application of new coatings during the ripening and storage of two types of Italian cheese and winter melon by using near infrared spectroscopy and aquaphotomics. The authors conclude that each coating generated a specific fingerprint for the same product suggesting that aquagrams could be a useful procedure for distinguishing the effects of different coating materials. A lot of publications related to aquaphotomics and monitoring of changes in biological systems under various perturbations can be found (Bázár et al., 2014; Gowen et al., 2015; Kovacs et al., 2016).

The aim of this paper is to demonstrate the feasibility of NIR spectroscopy and Aquaphotomics approach for rapid discriminating of wild and raised in the recirculation system perch, fresh and spoiled meat, freezing and thawing meat and quality defects of meat.

2. Materials and methods

2.1. Anayzed samples

2.1.1. First experiment

Thirty four perches (*Perca fluviatilis L.*) with a live weight of 120–140 g were investigated. Half of the fish were raised in the recirculating system of the experimental base of Agricultural Faculty of Trakia University, and the other half of the fish were caught with a fishhook at a Zhrebchevo Dam, Bulgaria). All the fish were killed by putting them in ice immediately after the catch, filleted and subjected to chemical and NIRS analysis.

Fish samples were prepared according to AOAC (2000; method 983.18) and subjected to determination of water content using air drying. Crude protein content was calculated by converting the nitrogen content, quantified by Kjeldahl's method, using an automatic Kjeldahl system (Kjeltec 8400, FOSS, Sweden). Lipid content was determined by the method of Soxhlet, using an automatic system (Soxtec 2050, FOSS, Sweden). Ash content was investigated by incineration in a muffle furnace (MLW, Germany) at 550°C for 8 h. Crucibles were equilibrated to the room temperature and weighed.

2.1.2. Second experiment

Chilled pork loin samples (12 different batches) were collected from different retail meat markets. The meat was cut in slices 1–1.5 cm tick, weighing 45–55 g, and placed in sterilized glass Petri dishes, in aseptic laboratory conditions. Samples were placed at a temperature of 6 ± 0,3°C for storage of 3, 7 or 10 days. Total bacterial count was determined according to ISO 4833 standard. Formed colonies in the agar are presented as $\log_{10}$CFU/g product.

2.1.3. Third experiment

Meat samples were collected from 72 cross-breed pigs, each with an approximate live weight of 115 kg. The pH of muscle samples was measured directly at 45 minutes post-mortem. Carcasses were divided into two classes according to pH values: normal meat with pH_{45} values higher than 5.8, and Pale, Soft, Exudative (PSE) meat with pH_{45} values lower than 5.8. Porcine muscle (*Longissimus thoracis et lumborum*) samples were taken 24 h after slaughter and divided into two parts. One part of each sample was scanned immediately, and a second part packed and sealed in a plastic bag to be deep frozen at –32°C for 6 h and kept at –21°C. Samples were thawed after one month and measured again.

2.2. NIR measurements and analysis

Diffuse reflection spectra of all tested food samples were obtained with a portable scanning NIRQuest 512 instrument in the range 900–1700 nm, using a reflection fiber-optic probe (Figure 1). For each sample measurements were made in 5 different points on the meat surface and then averaged. The spectral data processing and multiple scatter correction was carried out by Pirouette ver. 4.5 (Infometrix, Inc., Woodinville, WA, USA) software.

A radar chart called aquagram was used to display normalized absorbance values at several water bands on the axis originating from the centre of the graph. Water matrix coordinates at 1344, 1364, 1372, 1382, 1398, 1410, 1438, 1444, 1464, 1474, 1492 and 1518 nm, were used for axes. The values for aquagram Aq_λ are calculated using the following equation:

$$Aq_\lambda = \frac{A_\lambda - \mu_\lambda}{\sigma_\lambda},$$

where A_λ *is* absorbance after multiplicative scatter correction (MSC), μ_λ is the mean value of all spectra, and σ_λ is standard deviation of all spectra at wavelength λ, respectively.

2.3. Statistical analysis

Program STATISTICA, StatSoft, Inc., Tulsa, USA was used for statistical analysis of the data.

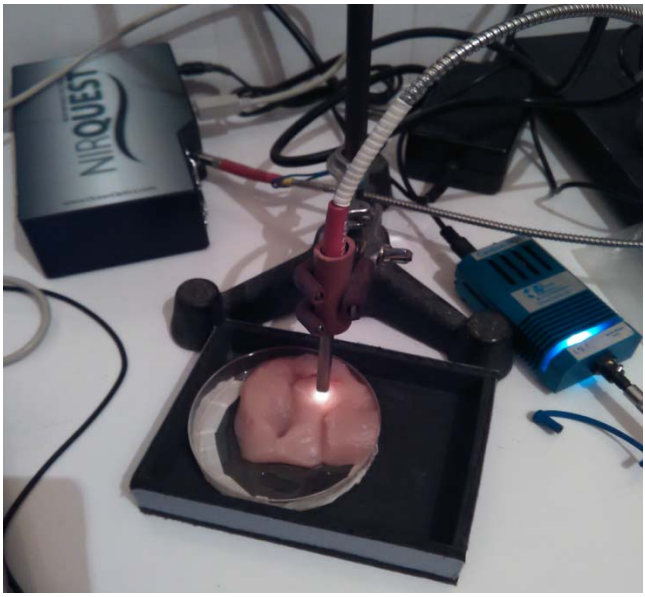

Figure 1. Measuremet of meat samples using a reflection fiber-optic probe and portable scanning NIRQuest 512 instrument

3. Results and Discussion

3.1. Experiment 1

Chemical composition of meat from wild perch (*Perca fluviatilis L.*) and the one raised in the recirculation system is presented in Table 1. The water content in the meat of wild perch is significantly higher than that in raised perch. The content of dry matter, crude protein and

crude lipids was higher in samples from perch raised in the recirculation system compared to these caught in Zhrebchevo Dam, Bulgaria. The ash content in the meat of wild perch was significantly less than the values in the cultivations in the recirculation system.

Table 1. Chemical composition of meat from wild perch (*Perca fluviatilis L.*) and the one raised in the recirculation system

Parameter	Raised perch	Wild perch
Water, %	77.07±0.03	79.70±0.07***
Protein, %	20.68±0.07	18.96±0.00***
Lipids, %	0.74±0.03	0.11±0.01***
Dry matter, %	22.93±0.03	20.30±0.07***
Ash, %	1.52±0.07	1.24±0.07*

*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$.

Differences in NIR spectra of meat from wild perch and the one raised in the recirculation system were observed (Figure 2). The most significant differences were observed at 960 nm, 1100 nm, 1160 nm, and in the region from 1254 to 1321 nm, 1400–1424 nm, and at 1582 nm, respectively. The most of absorption maximums, at which significant differences between spectra of two kind of perch occured (at 960, 1400, 1421 nm and 1582 nm), might be assigned to vibration of O-H group of water (Workman and Weyer, 2008).

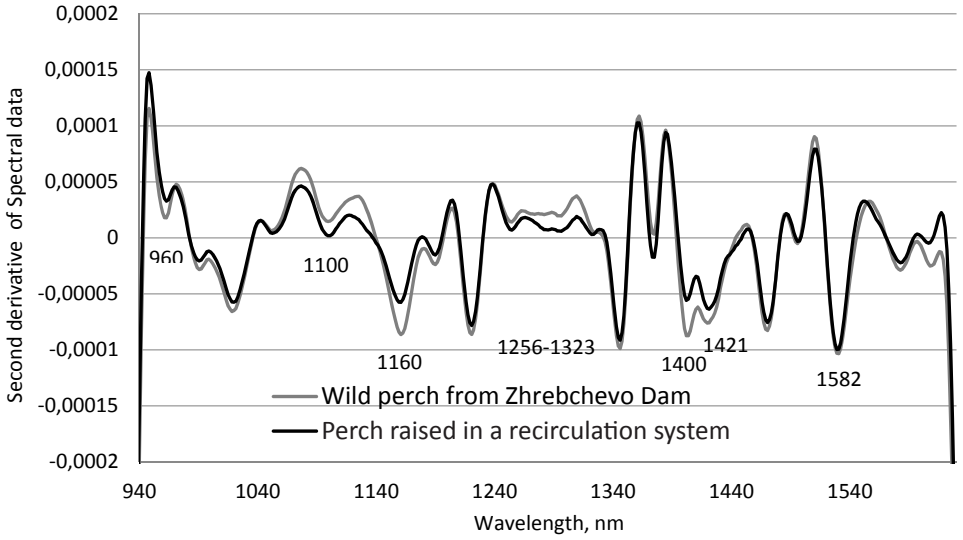

Figure 2. Second derivative absorbance spectra of meat from wild perch (*Perca fluviatilis L.*) and the one raised in the recirculation system

S o u r c e: Authors' own elaboration based on the research.

The aquagram, calculated using WAMACS coordinates, based on the spectral data of tested fish samples, is presented in Figure 3. The aquagram more clearly showed differences in NIR spectra of wild perch and the one raised in the recirculation system, caused by different chemical composition.

3.2. Experiment 2

The number of bacteria cells of meat samples during the investigated period varied between 3.146 to 9.505 $\log_{10}$CFU per 1 g product. Meat samples immediately after purchase had bacteria cells from 1.6×10^4 to 5.7×10^5 per 1 g. The number of bacterial cells increased to 10^8–10^9 per 1 g after 7 or 10 days storage period. These results indicated that the chilled pork samples have undergone a gradually microbiological spoilage during storage. Meat samples were classified as fresh, semi-fresh or spoiled, according to measured $\log_{10}$CFU/g values: lower than 4—fresh, values between 4 and 6—semi-fresh, values bigger than 6—spoiled.

Bacteria growing is connected with degradation of protein and carbohydrate from substrates and presence of proteins and possibly free amino acids, amines or peptides and their interaction with water. Such changes are recognized as the main indicator for the starting of spoilage in meat and meat products. Differences in absorption spectra of fresh, semi-fresh and spoiled meat were observed, most significant in 1300–1550 nm region. The absorbtion in that region might be assigned to vibration of O-H group of water and N-H stretching of proteins (amines and amides) and their interaction with water.

Spectral data of all samples with bacteria cells count lower than 3 $\log_{10}$CFU per 1 g were averaged. This procedure was repeated for samples with values from 3 to 4 $\log_{10}$CFU per 1 g, from 4 to 5 $\log_{10}$CFU per 1 g, etc. The averaged spectra were used for calculation of aquagram coordinates (Figure 4). Aquagram showed changes in meat during the spoilage process, connected with proportion of free and bounded water and interaction of products of proteolysis in meat with water.

3.3. Experiment 3

PSE meat occurs when the pigs suffer acute stress before slaughter. When there are sufficient energy reserves in the muscles, the initial pH can drop very fast post-mortem due to the production of lactic acid. This pH drop causes a denaturation of the myosin and sarcoplasmic proteins and results in pale meat with a low water holding capacity.

Lean muscle contains approximately 75% water. Water in meat existed in form of bound, entrapped and free water. Bound water is the water that exists in the vicinity of non-aqueous constituents (like proteins) and has reduced mobility. Entrapped water is another fraction of water that can be found in muscles. It can be easily converted to ice during freezing. Free water is defined as the water whose flow from the tissue is unimpeded. During freezing and thawing of meat, ice crystal growth causes biochemical and physical changes. The latter result in the disruption of cellular organelles and release of their contents into the meat drip juice. These changes lead to changes in water content and proportion of free and bounded water, change of pH, water-holding capacity, protein denaturation, texture and tenderness of

the meat (Vieira et al., 2009). At the micro-level, changes lead to oxidative processes and oxidation of lipids and proteins in destroyed cells (Xia et al., 2009).

Differences in absorption spectra between both normal and PSE meat and fresh and frozen then thawed meat were observed in the same region between 1350 and 1500 nm as for fresh and spoiled meat. These differences could be explained with changes in water content and proportion of free and bounded water in meat. This statement is confirmed by aquagrams (Figures 5 and 6) showing changes of water structure in normal and PSE meat, as well as between fresh and frozen meat.

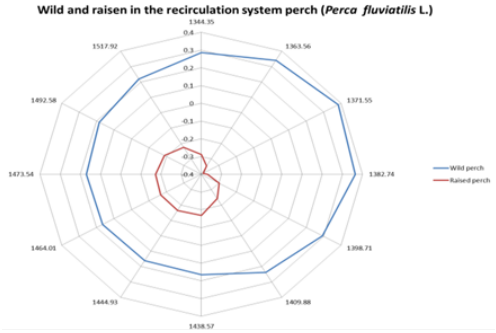

Figure 3. Aquagrams of wild perch (*Perca fluviatilis L.*) and the one raised in the recirculation system

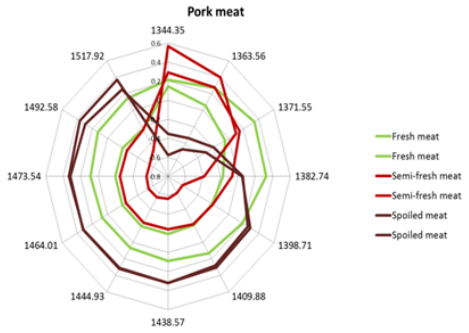

Figure 4. Aquagrams of fresh, semi-fresh and spoiled pork meat

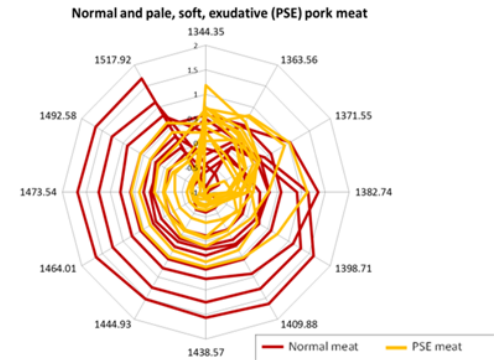

Figure 5. Aquagrams of normal and pale, soft, exudative pork meat

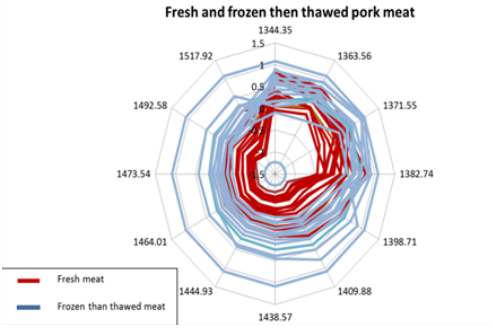

Figure 6. Aquagrams of fresh and frozen then thawed pork meat

Source (Figures 3–6): Authors' own elaboration based on the research.

4. Conclusions

In all of examined cases (normal and PSE meat; fresh and frozen then thawed meat, fresh and spoiled meat, meat of wild and caught in dam perch) differences in NIR in 1300–1550

nm region occurred, connected with water absorptions. These differences were clearly illustrated in obtained aquagrams.

Differences in absorption spectra and respective aquagrams of tested samples could be explained with functionally different structures of water in different kind of meat.

Aquagrams for all tested food products could be used for investigating differences, connected with quality of meat, degree of spoilage of meat, and type of fish. These results demonstrate the potential of portable fiber-optics near-infrared spectrometer for monitoring food quality through observation of water absorbance bands.

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Innowacyjne metody inżynieryjne w ocenie jakości i bezpieczeństwa żywności

Abstrakt: Wzrost poziomu aktywności i jakości życia człowieka zależy od wielu czynników. Jednym z nich jest bezpieczeństwo i wysoka jakość dostarczanej żywności. Postęp, jaki dokonał się w obszarze technologii, technik pomiarowych i narzędzi przetwarzania danych, umożliwił rozwój innowacyjnych metod inżynieryjnych dających gwarancję wysokiej skuteczności kontroli jakości żywności i zdrowia publicznego. W artykule przedstawiono analizę spektralną bliskiej podczerwieni i akwapotomikę jako nieinwazyjne i szybkie metody oceny jakości żywności przez obserwację pasm absorpcji wody.

Słowa kluczowe: spektroskopia bliskiej podczerwieni, akwapotomika, wielowymiarowa analiza danych, bezpieczeństwo i kontrola żywności