Online Version ISSN: 1314-412X
Volume 5, Number 1
March 2013

AGRICULTURAL SCIENCE AND TECHNOLOGY

2013

An International Journal Published by Faculty of Agriculture,
Trakia University, Stara Zagora, Bulgaria

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AGRICULTURAL SCIENCE AND TECHNOLOGY, VOL. 5, No 1, pp 115 - 120, 2013

Product Quality and Safety

Evaluation of pork meat quality and freshness using colorimetric and spectral methods

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Abstract. *The aim of the study was investigation of the feasibility of colour measurements and near infrared (NIR) spectroscopy as a tool for the prediction of pork meat freshness. Chilled pork loin samples (12 different batches) were collected from different retail meat markets. The meat was cut in slices 1-1,5 cm thick and placed in sterilized glass Petri dishes, in aseptic laboratory conditions. The samples were placed in cooling incubator for storage at 6°C for 10 days. On the day of samples preparing, as well as on the 3, 7 and 10 day during storage meat samples from each batch were taken for measurement. Biochemical and microbiological parameters - pH, amino acid nitrogen and total bacterial count were determined. Colour measurements were made by portable colorimeter Lovibond RT and data were presented as three-dimensional coordinates L*, a* and b* in the colorimetric system CIELab. NIR measurements were performed by NIRQuest 512 spectrometer in the region 900-1700 nm using reflection fibre-optic probe. Partial least square regression with internal cross-validation was used for calibration models development for determination of tested parameters on the base of spectral data.Differences in both colour coordinates and near-infrared spectral data of fresh and spoiled meat samples were found. Colour measurements of meat samples in our experiment did not allow accurate determination of parameters, characterizing meat spoilage. The most significant spectral differences were observed in the region from 1360 to1470 nm and at 1642nm. Determination of pH, amino acid nitrogen and total bacterial count by PLS regression on the basis of near-infrared spectra showed good accuracy of determination for pH and amino acid nitrogen content and very good accuracy of determination of total bacterial count. The results demonstrated that the NIR spectral measurement is superior to colour measurement for predicting microbial contamination and meat spoilage.*

Keywords: pork meat, quality, spoilage, colour, near–infrared spectroscopy

Introduction

Meat consumption has various traditions associated with different cultures. Raw meat, meat cuts, pre-packed meat and meat products have very different shelf-life. Immediately after obtaining in the slaughterhouse, the initial microflora of red meat carcasses ranges from 102 to 105 CFU/cm2, mainly composed by Pseudomonas spp., Moraxela spp., Psychrobacter spp, Acinteobacter spp. and members of family Enterobacteriaceae. The meat will undergo spoilage, which is a term for decomposition of organic matter under the action of a wide range of microorganisms – bacteria, moulds and yeasts, even if kept in refrigerated conditions. *Pseudomonas* spp. are aerobic psychrotrophic microbes and usually dominate in the spoilage microflora on meat in refrigeration temperatures, moreover *Lactobacillus* is the major anaerobic dominator (Gill and Newton, 1978; Zhang et al., 2012; Ercolini et al., 2009). The processes described are often supported by enzymes of the meat. Visible signs at the beginning of the spoilage process are changes on the meat surface. Usually meat color becomes darker, then yields to a bright greenish. In-depth color is usually darker. The surface of the meat changes from dry to moisten and with slime formation. At higher initial contamination slime develops more quickly and at a temperature above 10°C it may occur for several hours. Off-odors, off-flavors and undesirable appearance in the taste and texture are detectable changes in the process of deterioration. Accumulated bacterial metabolites resulting from the utilization of meat carbohydrates, protein and fat could be assumed as a hazard to the health of consumers.

Visible product characteristics as colour, surface moisture and slime, texture and smell are usually used as first indicators for meat freshness. Laboratory diagnosis and accurate evaluation of meat freshness and degree of spoilage is carried out by classic methods for pH, amino ammonia nitrogen, the presence of H_2S, NH_3, presence of biogenic amines, total bacterial count determination. These methods are time and labor consuming. Developing new, fast and reliable methods for rapid determination and indicating quality or safety of the meat are requirements of practice and business operators along the chain from the slaughterhouse to the meat shop.

Visual evaluation of meat color uses three properties – perception of lightness, saturation and tone type. Various authors have investigated the relationship between color coordinates and the quality of meat and meat products (O'Sullivan et al., 2003; Genchev et al., 2008). Most authors use the color space coordinates for detection of different quality problems in meat as pale, soft and exudative meat (PSE); red, soft and exudative (RSE); dark, firm and dry (DFD) (Xing et al., 2007). Some authors studied also the changes in color during storage. Jakobsen and Bertelsen (2000) examined the relationship between color and level of surface lipids oxidation in beef stored for 10 days. Authors found a reduction in redness (color parameter a*) in the process of storage and negative statistical dependence between this parameter and the level of lipid oxidation. Esmer et al. (2011) found that the color chromatic coordinates a* and b* for ground beef decreased during storage. They located significant dependence between these two color coordinates, which shows that the loss of redness (reduction of a*)

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and increase of browning are result of metmyoglobin formation which finally reduces the value of b*. Rosenvold and Wiklund (2011) also found that the color of chilled lamb changes during storage and the meat becomes less red and yellow (reduction in the color coordinates a* and b*).

Near infrared spectroscopy (NIRS) is a rapidly developing non-destructive method with multiple applications in the analysis of meat and dairy products. 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 (Sun, 2009). Successful applications of NIRS for determination of chemical composition of meat and some sensory characteristics of meat have been reported in literature (Prevolnik et al., 2004; Berzaghi and Riovanto, 2009). Several authors investigated possibilities of near-infrared and Infrared spectroscopy for determination of meat spoilage. 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. Aleksandrakis et al. (2009) analyze poultry meat stored 14 days at 4°C, using spectral analysis in the near-infrared region. The authors found that it is possible to distinguish samples on the first day from those stored 8 or 14 days on the basis of spectral data. The main distinguishing factor was increase of free amino acids and peptides in the muscles. Prado et al. (2011) investigate the potential of spectroscopy in the range from 1600 to 2400 nm and a portable spectrometer for determination of meat shelf-life by directly measuring the meat surface through the packaging foil on 1, 3, 5, 7, 9, 12 and 15 days of storage. Results showed that spectra of meat acceptable or not for consumption have market differences around 1660 nm. The developed NIRS model makes possible on-site prediction of the microbiological status of pork meat with a standard error of cross-validation of 1 logCFU/g.

The aim of this work was to investigate the feasibility of colour measurements or near-infrared spectroscopy for prediction of pork meat freshness and to compare their accuracy.

Material and methods

Meat samples

Fresh chilled pork meat (12 batches) originating from different slaughterhouses was purchased from a retail store. The meat has no additives as water, saline or brine mixtures. In aseptic laboratory conditions the meat was cut into slices 1-1,5 cm thick, weighing 45-55 g, and placed in individual sterile Petri dishes. Petri with meat samples were wrapped with transparent polythene foil to prevent moisture loss from the sample during storage. Samples were placed at a constant temperature of 6 ± 0,3°C (cooling thermostat camera TLSO 80/80 Etna, Bulgaria) for storage of 3, 7 or 10 days. In 3 days' interval samples were tested for: amino ammonia nitrogen, total bacterial count, pH, color index (color space coordinates) and spectral data in the NIR region.

Amino ammonia nitrogen

From each meat sample 25 g was cut and homogenated with 50 ml of distilled water. Homogenate was transferred to a volumetric flask and diluted to 100 ml with distilled water. After 5 min shaking, to 40 ml filtrate 25 ml of 10% solution of aluminum alum and 20 ml of saturated solution of barium hydroxide was added to precipitate the proteins. After filtration to 20 ml was added 0,3 ml indicator (neutral red and methylene blue) and titrated with 0,1 n sodium hydroxide up to color transition from purple to green. In the same flask 10 ml of formalin and 0,5 ml indicator was added (blue thymol blue with phenolphthalein). Titrated again with 0,1 n sodium hydroxide to color change from light green to violet. For control the same procedure was done as the meat sample was replaced with distilled water (25 ml). Amino ammonia nitrogen content is calculated by the equation $AA = 70. (V–V_1)$, where V and V_1 are the amount of 0,1 n sodium hydroxide consumed in the titration of meat filtrate and control.

Total bacterial count

Detection of total bacterial count was carried out according to standard ISO 4833 including sample preparing, 10 fold serial dilutions, inoculation in Plate count agar. From each meat sample 10 g was cut with sterile instruments in Stomacher bag. MRD (Maximum recovery diluents, Merck) was added in aliquot of 90 ml and homogenized (Stomacher 400) at 256 rpm-1 for 60 seconds. From the homogenate decimal dilutions were prepared up to level 10^{-5}. And inoculums of 1 ml were transferred to empty sterile Petri dish and poured over with cooled up to 45 °C PCA agar. All inoculated petri dishes were incubated at 30 °C for 24-48 hours. Formed colonies in the agar are presented as CFU/g product or $\log_{10}$CFU/g product.

pH

Measuring the pH of the samples was performed with a laboratory pH meter (PB-11, Sartorius).

Color space coordinates SCIELab

Color characteristics of the samples were measured with a portable colorimeter Lovibond RT series (Tintometer Ltd, Dortmund, Germany). For each sample measurements were made in 10 different points on the sample surface and then averaged. Data are presented as three-dimensional coordinates in the colorimetric system CIELab, as L*, a* and b* corresponding to the range of white-black, green, red and yellow-blue, respectively.

NIR Spectral measurements

Diffuse reflection from the sample surfaces were obtained with a scanning spectrophotometer NIRQuest 512 (Ocean Optics) in the range 900-1700 nm. For each sample measurements were made in 5 different points on the sample surface and then averaged. The resulting spectral data were used for further analysis by Pirouette 2.02 (Infometrics, Inc., Woodinville, WA, USA) for quantitative analysis using Partial Least Square Regression (PLS).

Statistical analysis

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

Results and discussion

Biochemical and microbiological measurement

Mean values and standard deviation of biochemical and microbiological parameters, measured at 0, 3, 7 и 10 day of storage are presented at Table 1. The values of pH varied from 5.72 to 6.91. The biggest variations in the values, measured on the same day, were found for amino acid nitrogen (AAN) content. Among all the samples, AAN ranged from 35 to 210 mg/100g. Values of AAN increased during the storage, except for some samples kept for 7 or 10 days. The possible reason for that could be evaporation of some volatile products, resulting from meat spoilage. Meat with amino acid

Table 1. Mean values and standard deviation of pH, amino ammonia nitrogen (AAN) and total bacteria count (log_{10}CFU/g product), measured at 0, 3, 7 and 10 day of storage.

Parameter	0 day	3 day	7 day	10 day
pH	6.12 ± 0.28	6.01 ± 0.28	6.09 ± 0.16	6.04 ± 0.03
AAN, mg/100g	99.17 ± 39.24	155.75 ± 25.87	119.00 ± 56.24	137.67 ± 17.62
logCFU/g	4.88 ± 0.80	7.29 ± 0.46	8.63 ± 0.53	8.52 ± 0.58

nitrogen content lower than 80 mg/100g is considered fresh, meat with amino acid nitrogen from 80 to 130 mg/100g – semi-fresh and with values higher than 130mg/100g – spoiled, respectively.

The number of bacteria cells varied between 3.146 to 9.505 logCFU per 1 g product. Meat samples immediately after purchase had bacteria cells from $1.6x10^4$ to $5.7x10^5$ 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 gradual microbiological spoilage during storage. Meat samples with values of log_{10}CFU/g lower than 6 were classified as fresh or semi-fresh. Samples with values of log_{10}CFU/g bigger than 6 were classified as spoiled.

The three investigated parameters for evaluation of meat quality (pH, AAN and logCFU/g) didn't change synchronously for all tested samples. A different number of samples was classified as fresh or spoiled according to different criteria.

Colour measurement

Mean values and standard deviation of colour coordinates L*, a* and b*, measured at 0, 3, 7 and 10 day of storage are presented in Table 2. Colour coordinates a* and b* initially increased to 7 day of storage and decreased at 10 day of storage. Decreasing of colour coordinates a* and b* showed that meat becomes less red and yellow and more brown. There were significant differences in initial colour coordinates of meat samples. For example, coordinate L* varied from 46.93 to 58.60 at 0 day and from 47.80 to 60.54 at 7 day. Similar observations were found for other parameters a* and b*. Investigated meat samples originated from different slaughterhouses and different retail stores. It is very difficult to distinguish changes, caused by animal breed, shop condition etc. from changes connected with storage and spoilage processes.

The relationship between colour information and pH, AAN and microbial counts was calculated. The correlation coefficient or coefficient of multiple correlations between pH, AAN or total bacteria count and colour coordinates are presented at table 3. The highest correlation between parameters, characterizing meat spoilage and colour coordinates were found for L* – correlation coefficient of -0.417 for pH, 0.368 for AAN and 0.273 for logCFU/g. Significant correlation was found between colour coordinate a* and pH and colour coordinate b* and logCFU/g. The coefficient of multiple correlation between all colour coordinates and pH, AAN or microbial counts varied from 0.359 and 0.439. The relationship between all colour coordinates and the investigated parameters for evaluation of meat quality was statistically significant, but standard error of estimation resulting from the obtained regression equations was high and very close to the standard deviation of the data. For example, the standard error of estimation of logCFU/g was 1.58 while the standard deviation of the measured values was 1.70. Colour measurements of meat samples in our experiment did not allow accurate determination of parameters characterizing meat spoilage.

Near-infrared spectral characteristics of fresh and spoiled samples

Differences in absorption spectra were observed between fresh and spoiled meat. Spectra of meat sub-samples, measured at 0, 3 and 7 day after purchasing and transformed as second derivative are presented on Figure 1. Although a similar pattern was observed in the spectral information extracted from both samples, some difference in the magnitude of absorbance existed between spectra. The most significant differences were observed in the region from 1360 to1470 nm and at 1642 nm. The absorption maximum at 1411 and 1465 nm 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 (Workman and Weyer, 2008). The absorption at 1642 nm could be connected with C-H group. Additionally small spectral changes were observed at 986, 1048, 1096, 1160 and 1254 nm.

Table 2. Colour coordinates (CIELab L*a*b*), measured at 0, 3, 7 and 10 day of storage at 6±0.3 °C.

Colour coordinate	0 day	3 day	7 day	10 day
L*	52.39 ± 2.1	54.98 ± 2.6	55.49 ± 1.9	58.02 ± 4.0
a*	2.37 ± 0.7	2.83 ± 0.9	3.09 ± 0.6	1.84 ± 0.7
b*	13.00 ± 0.7	14.12 ± 0.8	14.70 ± 1.0	11.70 ± 2.5

Table 3. Correlation coefficient or coefficient of multiple correlations between pH, AAN or total bacteria count and colour coordinates.

Parameter	L*	a*	b*	L*+a*+b*
pH	-0.417***	-0.227***	-0.126	0.436***
AAN,	0.368***	-0.133*	0.091	0.358***
logCFU/g	0.273***	0.127	0.255***	0.439***

*- statistically significant at $p<0.05$; *** - statistically significant at $p<0.001$.

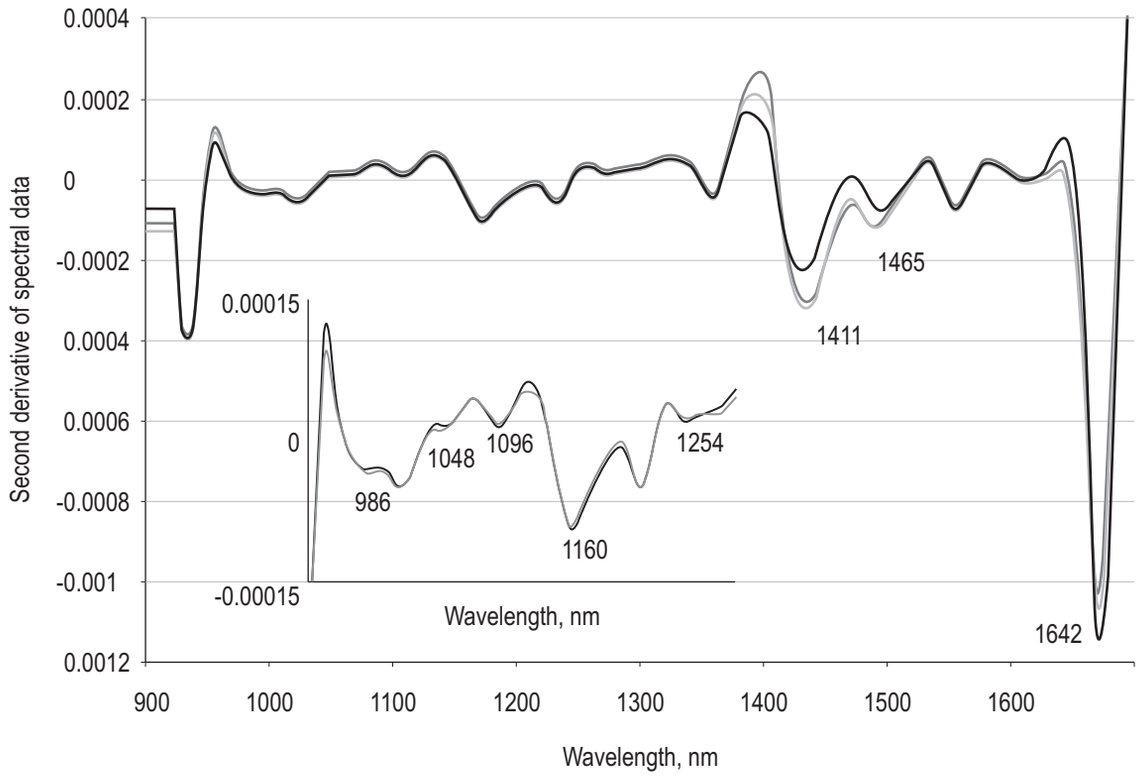

Figure 1. Spectral data of meat subsamples, measured at 0, 3 and 7 day after purchasing, transformed as second derivative. (blue – 0 day, red – 3 day, green – 7 day).

Similar important wavelengths at which the fresh and spoiled pork samples could be distinguished (964 nm, 1128 nm, 1151 nm, 1301 nm, 1341 nm, 1395 nm and 1635 nm) were reported by Barbin et al. (2012). Prado et al. (2011) also found that spectra of pork meat acceptable or not for consumption have marked differences around 1660 nm. Bacteria, either pathogens or not, use mainly protein and carbohydrate from substrates to maintain their vital functions or for replication and bacterial biomass augmentation. These bands strongly suggested that the differences in NIR spectra of fresh and spoiled meat are connected with the presence of proteins and possibly free amino acids, amines or peptides and their interaction with water. Such an observation is consistent with the proteolytic changes recognised as a major event occurring during microbial spoilage. and it could suggest the occurrence of proteolytic changes, which are recognized as the main indicator for the start of spoilage in meat products (Alexandrakis et al., 2009).

Quantitative determination of pH, AAN and microbial counts on the basis of spectral data.

PLS regression was used for quantitative determination of pH, AAN and microbial counts on the basis of spectral data. The calibration equations for each parameter were developed and validated with leave-one-out cross validation. The leave-one-out method is recommended when a few samples are used to build the calibration equations. Cross-validation calculates the predictive ability of potential equations to help determine the appropriate number of components (latent factors) in the model. The leave-one-out cross-validation routine works by omitting one observation once at a time, recalculating the equation using the remaining data, and then predicting the omitted observation. This routine is repeated until each observation in the dataset is used once as validation data. Statistical data of the calibration equations for NIRS prediction of the tested parameters are presented in Table 4.

The results of the quantitative determination of pH, amino acid nitrogen content and logCFU of meat samples by PLS regression are presented in Table 4. Figures 2-4 graphically illustrate the relationships between actual and NIR spectroscopy predicted parameters of the tested pork samples. The accuracy of each calibration equation was evaluated based on R – multiple correlation coefficients between values of meat parameters and NIR spectra, SEC - standard error of calibration, SECV – standard errors of cross validation and value of RPD - the ratio of standard deviation of data set SD to the SEC. The RPD evaluated the prediction errors in light of the standard deviation of the reference data and thus enables comparison between models for constituents with different variation ranges. The RPD values showed levels of prediction accuracy as

Table 4. Statistical data of the calibration equations for NIRS prediction of tested parameters in examined meat samples.

	SECV	Rcv	SEC	Rcal	RPDcv	RPDcal
pH	0.11	0.82	0.08	0.84	1.64	2.25
AAN, mg/100g	27.28	0.77	22.5	0.82	1.68	2.04
logCFU/g	0.63	0.84	0.52	0.86	2.70	3.27

R – multiple correlation coefficient between values of meat parameter and NIR spectra; SEC - standard error of calibration; SECV – standard errors of cross validation; RPD - the ratio of standard deviation of data set SD to the SEC or SECV.

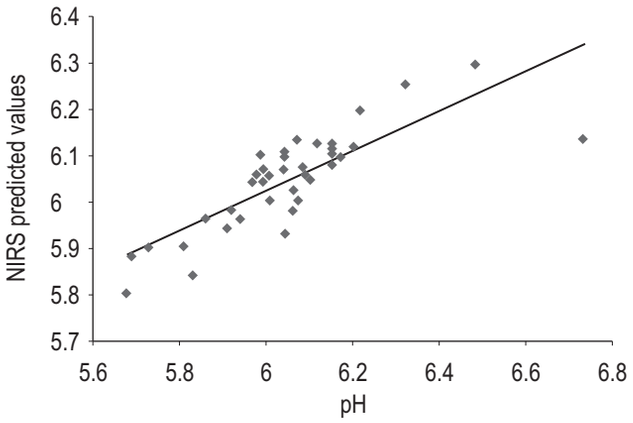

Figure 2. Near-infrared predicted versus measured pH values of tested pork samples using PLS model.

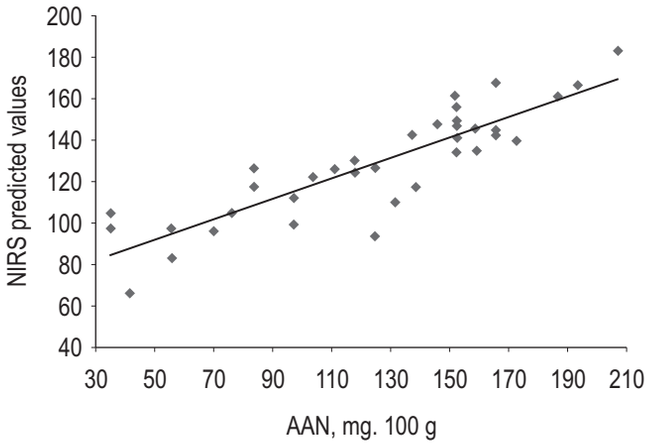

Figure 3. Near-infrared predicted versus measured amino ammonia nitrogen content of tested pork samples using PLS model.

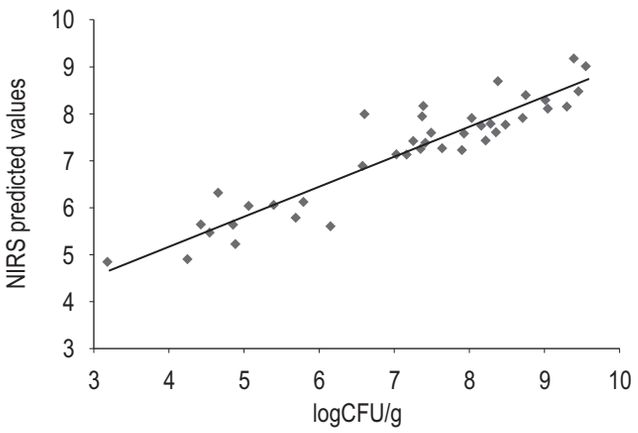

Figure 4. Near-infrared predicted versus measured logCFU/g of tested pork samples using PLS model.

follows: RPD between 2.0 and 2.5 indicates good prediction; RPD between 2.5 and 3.0 indicates very good prediction; and RPD >3 indicates excellent prediction. According to these criteria, determination of logCFU/g was very good. The obtained accuracy of determination of logCFU/g is similar to results reported by Horvat et al. (2008) in sliced pork meat – a correlation coefficient of 0.977, and a root mean square error of prediction (RMSEP) 0.438 log colony forming units/g and Lin et al. (2004) in chicken breast muscle – R = 0.91 and SEP = 0.48 log CFU/g. The RPDcal was higher than 2 for determination of pH and AAN which showed good accuracy of determination.

In general, the results from spectral analysis are considerably better than those obtained from the three colour parameters (L*, a* and b*) with correlation coefficients between 0.77 and 0.84. The microbial growth could cause several changes in the samples other than colour alterations, which are not clearly detected using such colour coordinates. The good agreement obtained from the NIR region and logCFU/g could be possibly due to changes in organic components, resulting from the bacterial degradation of meat. Computer vision technology is valuable when quality attributes of a product are related to its extrinsic characteristics, as it was the case with possible colour changes during the spoilage process. However, this technique becomes less useful or ineffective when quality attributes are mainly determined by the intrinsic properties of the product, such as composition and biochemical changes, which are not easily detectable based on the colour of the surface. The results demonstrate that the NIR spectral measurement is superior to colour measurement for predicting microbial contamination and meat spoilage.

Conclusion

Differences in both colour coordinates and near-infrared spectral data of fresh and spoiled meat samples were found. Colour measurements of meat samples in our experiment did not allow accurate determination of parameters characterizing meat spoilage.

The most significant spectral differences were observed in the region from 1360 to1470 nm and at 1642 nm. The results of quantitative determination indicated the suitability of near-infrared spectroscopy for the determination of pH, amino acid nitrogen content and bacterial load of meat samples in a non-destructive and rapid way. The model for logCFU/g resulted in high correlation coefficients of 0.84 and SEC and SECV of 0.52 and 0.63 log10 CFU g^{-1} for calibration and cross validation, respectively.

The potential of NIRS for pork meat qualification was demonstrated. Fibre-optics NIR instrument could be used for fast discrimination between fresh and spoiled pork meat. The method is rapid and non-destructive, without sample preparation before measurement. The application of NIR spectroscopy can improve analysis of meat quality through the optimised laboratory efficiency, and the tighter production control.

Acknowledgments

Financial support for the study was provided by the National Scientific Fund of the Bulgarian Ministry of Education, Youth and Science, project DO -02/143.

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Product Quality and Safety

Instruction for authors

Preparation of papers

Papers shall be submitted at the editorial office typed on standard typing pages (A4, 30 lines per page, 62 characters per line). The editors recommend up to 15 pages for full research paper (including abstract references, tables, figures and other appendices)

The manuscript should be structured as follows: Title, Names of authors and affiliation address, Abstract, List of keywords, Introduction, Material and methods,Results, Discussion, Conclusion, Acknowledgements (if any), References, Tables, Figures.

The title needs to be as concise and informative about the nature of research. It should be written with small letter /bold, 14/ without any abbreviations.

Names and affiliation of authors

The names of the authors should be presented from the initials of first names followed by the family names. The complete address and name of the institution should be stated next. The affiliation of authors are designated by different signs. For the author who is going to be corresponding by the editorial board and readers, an E-mail address and telephone number should be presented as footnote on the first page. Corresponding author is indicated with *.

Abstract should be not more than 350 words. It should be clearly stated what new findings have been made in the course of research. Abbreviations and references to authors are inadmissible in the summary. It should be understandable without having read the paper and should be in one paragraph.

Keywords: Up to maximum of 5 keywords should be selected not repeating the title but giving the essence of study.

The introduction must answer the following questions: What is known and what is new on the studied issue? What necessitated the research problem, described in the paper? What is your hypothesis and goal ?

Material and methods: The objects of research, organization of experiments, chemical analyses, statistical and other methods and conditions applied for the experiments should be described in detail. A criterion of sufficient information is to be possible for others to repeat the experiment in order to verify results.

Results are presented in understandable tables and figures, accompanied by the statistical parameters needed for the evaluation. Data from tables and figures should not be repeated in the text. **Tables** should be as simple and as few as possible. Each table should have its own explanatory title and to be typed on a separate page. They should be outside the main body of the text and an indication should be given where it should be inserted.

Figures should be sharp with good contrast and rendition. Graphic materials should be preferred. Photographs to be appropriate for printing. Illustrations are supplied in colour as an exception after special agreement with the editorial board and possible payment of extra costs. The figures are to be each in a single file and their location should be given within the text.

Discussion: The objective of this section is to indicate the scientific significance of the study. By comparing the results and conclusions of other scientists the contribution of the study for expanding or modifying existing knowledge is pointed out clearly and convincingly to the reader.

Conclusion: The most important consequences for the science and practice resulting from the conducted research should be summarized in a few sentences. The conclusions shouldn't be numbered and no new paragraphs be used. Contributions are the core of conclusions.

References:

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Todorov N and Mitev J, 1995. Effect of level of feeding during dry period, and body condition score on reproductive performance in dairy cows,IXth International Conference on Production Diseases in Farm Animals, Sept.11 – 14, Berlin, Germany, p. 302 (Abstr.).

Thesis:

Penkov D, 2008. Estimation of metabolic energy and true digestibility of amino acids of some feeds in experiments with muscus duck (Carina moshata, L). Thesis for DSc. Agrarian University, Plovdiv, 314 pp.

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AGRICULTURAL SCIENCE AND TECHNOLOGY

Volume 5, Number 1
March 2013

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