On site NIR spectroscopy to control the shelf life of pork meat

N. Prado[1], V. Fernández-Ibáñez[2], P. González[1], A. Soldado[2*]

[1]Research Association of Meat Industry. ASINCAR. 33180, Noreña, Spain

[2]Department of Animal Nutrition, Grasslands and Forages Regional Institute for Research and Agro-Food Development. SERIDA, 33300, Villaviciosa, Spain.

*Author to whom correspondence should be addressed (e-mail: asoldado@serida.org

Telephone number: +34 985890066. Fax number: +34 985891854

ABSTRACT

Control of meat shelf-life includes the time that it remains in the exhibitor of sale (such as the supermarket) until its rejection for the consumer, or withdrawal due to expiry date. Near infrared spectroscopy (NIRS) is one of the most promising techniques for large-scale meat quality control. This study investigated the potential of *on-site* NIRS portable instrumentation based models to predict three microbiological parameters to establish if pork meat is acceptable or not for consumption (aerobic *Mesophilous* microorganisms, *Enterobacteriae* and lactic acid bacteria) and pH to quality control food preservation and shelf life extension on intact slices of pork meat packaged under two different modified atmospheres. NIR calibrations were developed by using an *on-site* Phazir instrument (Polycromix,Wilmington, USA) in the range 1600-2400nm. A total of 252 samples of pork meat slices were directly scanned twice in reflectance mode on trays, once before and other one after removing the film cover at 1, 3, 5, 7, 9, 12 and 15 days of storage. Results showed that spectra of meat acceptable or not for consumption have marked differences around 1660 nm. NIRS quantitative prediction models showed r^2 values between 0.19-0.65 for the microbiological parameters assayed. The developed NIRS methodology makes possible on-site prediction of microbiological status of pork meat with and standard error of cross validation around 1 Log cfu/g, Results have shown that there are not influence of MAP (modified atmosphere packaging) on calibration statistics.

Key words: Near infrared reflectance spectroscopy, pork, meat, food preservation

INTRODUCTION

The meat industry is an important economic sector in most developed countries where the demand of meat product is high. Nowadays, food preservation is vital to safety, extending product shelf life, and maintaining quality attributes that customers find appealing. Meat may undergo proteolysis, lipolysis and enzymatic and/or chemical oxidation during refrigerated storage, leading to changes in organoleptic characteristics and perceptions[1].

The globalization of the food business, and the logistics of distribution from processing centers, makes it necessary to control shelf life of food as a result of microbial contamination, to avoid an increase in the risk of food-borne illness[2]. In checking shelf life it is also important to limit product and raw material wastage by the manufacturer, allowing efficient and low-cost food processing.

In recent decades, meat commercialization strategies have involved different conservation methods (freezing, vacuum packaging or modified atmosphere packaging (MAP)) to limit the growth of psychrophilic bacteria involved in the microbial spoilage of refrigerated animal foods[3]. Some reports have established that atmosphere changes can affect the microflora of the product usually resulting in shelf-life extension. Common gases used in MAP (CO_2, O_2 and N_2) inhibit bacterial growth, suppress aerobic growth, prevent anaerobic growth, retain meat color and avoid oxidation of fats and pack collapse[4].

Different techniques, such as, chemical procedures, instrumental/ microbiological and screening methods have been used to provide information about meat quality and safety. However, those techniques are destructive, time consuming and consequently unsuitable

for on-line/on-site application[5]. In contrast to conventional methods for the determination of meat safety parameters, near infrared reflectance spectroscopy (NIRS) shows considerable promise for non-destructive analysis of agro-food products, and it is one of the most promising techniques for large scale meat quality evaluation[6]. It has the great potential of predicting quickly and accurately different attributes of meat quality, it allows rapid and frequent measurements, and, it does not requires sample preparation.

Moreover, recent developments of NIR instruments have increased the suitability of NIR spectroscopy for predicting meat quality in the industry and in the market. An NIR instrument with a diode array detector is able to scan all wavelengths in a few milliseconds, allowing measurements over a large meat surface area. New generation NIRS instruments are much more versatile, more portable and better adapted to on-site, non-destructive measurement avoiding any modifying package or meat samples[7]. Previous studies[8] have estimated successfully beef colour on-line by direct application of a fiber-optic probe to the *M. longissimus thoracis* immediately after exposing the meat surface at quartering in the abattoir. However the major source of error when monitoring on-line intact meat samples is related with the fact that light penetrates only a few millimeters into the high water meat and thus, only a small fraction of the overall meat flow can be monitored . To solve this question it is necessary to take into account that the longer the measurements are recorded, the more representative the NIR measurements will be for the whole batch of meat.

Consequently there is a great interest among the food industry, retailers, consumers' rights and food safety controlling bodies in developing accurate, cost-effective, rapid,

reliable, non-invasive and non-destructive methods to evaluate real-time freshness of meat products.

The aim of the present work was to establish the predictive ability of on-site NIRS portable instrumentation to predict microbiological spoilage on intact slices of pork meat related with preservation conditions at the market level as an estimator of the shelf-life of the product.

MATERIAL AND METHODS

Preparation of samples and storage

A total of 9 pigs were slaughtered in three batches of 3 animals each on three different dates between December 2008 and February 2009. The pigs were reared by different local farmers and slaughtered. At 24 h post mortem a section of the carcasses, *longissimus dorsis* muscle were transferred to the processing plant to be filleted. Fillets were packaged in polyethylene trays under two different modified atmospheres treatments in replicate: Freshline™ 3MIX (Gas A) and Freshline™ 3MIX 20/5 (Gas B) (Air Liquide, Spain) containing 30/ 40/ 30 and 5/20/75 of $O_2/CO_2/N_2$ respectively. The vacuum traysealer used was a MULTIVAC T-200 (Germany) connected to the gas mixer outlet. All samples were kept under refrigeration at 4 ºC. For analysis sampling was carried out at 1, 3, 5, 7, 9, 12 and 15 days of storage. In summary the experimental design involved a total of 126 samples per Gas (9 pigs x 7 times x 2 replicates).

Microbiological Analysis

For these assays, pork trays were opened and a portion of fillets samples were transferred aseptically with a sterile tweezers into masticator bags, weighed and blended with 225 ml of sterile peptona water solution (0.1 %) in a stomacher (VWR International-Eurolab S.L.). Serial dilutions from the microbial extracts were prepared in peptone water. Aerobic *Mesophilous* microorganisms were investigated in plate count agar (PCA, Oxoid Ltd., London, UK) after incubation at 30ºC for 72 h (standard error= 0.30 log). Total *Enterobacteriaceae*, were determined by inoculation of 1.0 ml sample into 15 ml of molten (45ºC) violet red bile glucose agar (Oxoid code CM 485). After setting, a 10 ml overlay of molten medium was added and incubation was carried out at 37 ºC for 24 h. The large colonies with purple haloes were counted (standard error= 0.29 log). Lactic acid bacteria were determined on the Man Rogorosa Sharpe medium (Oxoid code CM361) after incubation at 25 ºC for 5 days. All plates were examined visually for typical colony types and morphological characteristics associated with each growth medium (standard error= 0.25 log).

In this study *Pseudomonas* or *Brochothrix thermosphacta* are not included because this research works is focused on those legislated parameters with high bacterial growth, to get a correlation between NIR spectra and reference data.

Physicochemical Analysis

pH was determined on two replicates in the central area of the fillets using a MP 120 Mettler -Toledo pH meter (Mettler Toledo, GMbH; 8603 Schwarzenbach, Switherland) fitted with a combined glass electrode (InLab 427) and previously calibrated at pH 4.0 and 7.0.

NIR spectroscopy Analysis

A representative calibration set of 252 samples of pork meat slices, 126 samples for each gas including samples acceptable and no acceptable for consumption, were directly scanned in reflectance mode, on the intact fillet using a portable near infrared analyzer from Polychromix PHAZIR™ (Wilmington, MA, USA), in the range between1600 and 2400nm. Spectral acquisition was carried out in direct contact analysis mode and each fillet was scanned twice, once before and once after removing the film closing the tray. Reflectance data were stored as log (1/R) at 8 nm intervals. Reflectance energy readings were referenced to corresponding readings from a ceramic disk. In order to have a representative spectral signature of the pork meat, the spectrum of each slice sample was the average of ten scans recorded in different points of the sample.

The software provided by Polycromix™, Phazir MG, v1.5.6.1 (Polychromix, USA) was used to collect and storage spectra. The WinISI II software v.1.05 (Foss-Tecator-Infrasoft International, Port Matilda PA, USA 2000) was used for computer operations and spectral data processing analysis.

To structure the calibration NIRS populations, an initial analysis of principal component (PCA) was performed to centre the population and to calculate the outlier spectra using the Mahalanobis distance (GH)[10].

The principles and procedures involved for qualitative analysis are essentially identical to those involved in quantitative analysis. In this study a calibration matrix with discrete variables was set up with values of one or two for meat fit for consumption or not respectively. The calibration models were performed by partial least square (PLS) regressing the wavelength data on the groups defined as categorical variables with one

or two; cross validation was used to test the accuracy of the model at each step. Quantitative predictive equations were developed using modified partial least square (MPLS) regression with internal cross validation and scatter correction using Multiplicative Scatter Correction (MSC) and Standard Normal Variate (SNV) and detrend transformations[11,12]. Cross validation was used to avoid overfitting of the equations and to estimate the prediction error by splitting the calibration set into groups. Four derivative mathematical treatments were tested to develop NIR calibrations: 0,4,4,1; 1,5,5,1, 2,5,5,1 and 2,4,4,1; where the first digit is the order of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in a running average or smoothing and the fourth is the second smoothing[13]. The pretreatments and math treatments detailed before have been assayed with all the parameters (aerobic *Mesophilous* microorganisms, Enterobacteriaceae, Lactic acid bacteria and pH), and the best prediction model was selected in base of its precision using the lowest standard error of cross-validation (SECV) and highest coefficient of determination in cross-validation (r^2).

RESULTS AND DISCUSSION

To explain the influence of reflectance bands we must to know the loadings (areas where differences in variance coincide with wavelengths known to be associated with molecular grouping and vibrations) and weights (degree to which the variance has actually been used in the computation factors across the wavelength range) for each factor of variance. When the weights are displayed across the wavelength range, peaks indicate the influence of molecular groupings responsible of wavelengths bands and the principal reflectance bands for common constituents of materials of plant and animal origin[15,16].

Figure 1 shows the near infrared mean and second derivative spectra of pork fillet samples (Gas A and Gas B) as log (1/R) using a infrared region in the 1600 to 2400 nm, grouped by packing under different controlled atmospheres before opening the package and it is very similar to those reported on fresh meat pork employing at-line instruments. The absorbance levels (Figure 1) attributed to the absorptions from O-H and C-H stretching vibrations, were similar to others reported by Prieto et al.[14] using a spectrophotometer model InfraAnalyzer 500 (Bran+Luebbe, GmbH, Norderst, Germany). In detail, the most significant absorption wavebands from 1780 nm to 1835 are related to the CH_2 stretch first overtone (related to fat content), absorbing at 1890-2000 nm with water absorption bands and indicates the absorption region of the O-H bonds and at 2250-2340 the absorption of C-H bonds related to fatty acids[15].

Our results suggest that some of the most important wavebands to establish correlation between the shelf life extension of pork meat and the spectral data showed strong absorbance in the water region, it is the dominant tissue chromophere in the NIR part of the spectrum. Water is a variable constituent in meat representing up to 60% of the total fresh matter[16]. The wavebands related with fat and water contents appear to have the most relevant bands in the NIR region for compositional assessment of meat[17] according previous reports.

Figures 1 and 2 show the mean spectra of pork meat samples acceptable and non-acceptable for consumption and packed with the two atmospheres assayed. The spectra obtained for samples preserved with gas A displayed considerable areas of differences in the regions around 1660 nm; 1755-1765 nm; 1850-1890 nm and 2348-2400 nm, these bands are related with C-H bonds of fat. Nevertheless, the spectra obtained for samples

packed with gas B revealed strong similarities between both extreme spectra (acceptable or not for consumption), with only marked differences around 1660 nm. This range around 1660 nm could be used to identify spectrally the deterioration and alterations that occur in microbiologically contaminated meat, but it should be studied.

The growth curves of the microorganisms tested for the controls carried out during the 15 days of the experiment are detailed in Figure 3. As can be seen, there are not differences between gas A and gas B. For aerobic *Mesophilous* microorganisms (legislated parameter) the threshold value has been established taking into account the limit of acceptability of shelf-life 6.699 log cfu/g and covering the range from acceptable for human consumption to unacceptable[18]. The range of variability of aerobic *Mesophilous* microorganisms was quite similar for both atmosphere treatments, between 2.85-9.58 log cfu/g for gas A and 3.04-9.50 log cfu/g for gas B. Samples with satisfactory microbiological parameters or unfit for human consumption were observed around ten days from packing. *Enterobacteriaceae* and lactic acid bacteria values were similar during sampling time for both gases[8].

As detailed in the Material and Methods, four mathematical treatments were attempted to develop NIRS calibration models able to predict microbiological parameters and pH. It is necessary to remark that, prediction of microbiological parameters was explored scanning intact samples before and after package opening.

Table 1 shows optimised results from mathematical treatments, and scatter corrections for each calibration equation using cross validation and employing spectra data collected after removing film and opening the package. The best equations for aerobic

Mesophilous microorganisms were obtained using first derivative math treatment for gas A, B and second derivative for A+B. In Figure 4 are shown the results of comparison between predicted and reference values. For *Enterobateriaceae* and lactic acid bacteria the math treatments were different depending on gas (A or B), obtaining the best coefficient of determination on cross validation (r^2= 0.420) when grouping database of both gases. The results of gas B were better than gas A, but with poor results for both gases. For lactic acid bacteria the statistics for gas A were better than gas B and gas A+B. As can be seen in Table 1, the maximum value for the coefficient of determination on cross validation was 0.65 with standard error close to ±1 log cfu/g for aerobic *Mesophilous* microorganisms. Statistical coefficients for pH showed similar results when comparing gas A and A+B (r^2= 0.47).

These correlation results, when developing calibration models using NIR Spectroscopy on reflectance mode, can be explained taking into account the following considerations: a) minced samples are more homogeneous than intact meat; b) in intact meat samples, the muscle fibres or myofibrils themselves may act as optical fibres tending to conduct NIR light along their length by a series of internal reflections, c) intact samples absorbs more energy therefore giving less reflectance when comparing with homogenized meat, d) homogenization severely disrupts the structure of the muscle destroying and randomizing the fibre arrangement of the muscle as well as averaging the effects of scattering by fibres[8]. In this context, the review of Prieto et al.[8] about application of NIR reflectance spectroscopy to predict meat and meat products quality explains the difference between results obtained in intact *vs.* minced meat samples.

With reference to the microbiological parameters evaluated in this work it is necessary to remark that there are no studies related to NIR prediction in intact pork meat by using portable instruments. However, previous researchers, using at-line FTIR/ATR instrument (range 2500-25000nm; Mid Infrared) have reported satisfactory results to predict qualitative and quantitatively counts of total viable bacteria (TVC) of beef fillets, combining artificial Neuronal Networks with spectra data[19], and with an accuracy factor around 1 log cfu. cm^{-2}. By using the same instrument (MIR, 2500-25000nm) Ammor et al.[20] demonstrated that the MIR spectra may be considered as a metabolic fingerprint able to monitor minced beef freshness stored under different storage conditions (packaging and temperature). But the most significative difference between these previous studies and our work is related with the instrumentation because in this study we have worked with a hand held portable and low cost Near Infrared (1600-2400nm) instrument able to be used at supermarket level as quality control system.

Regarding the prediction of pH value we have found no satisfactory statistic results. Although some authors[21] have related the reduced precision obtained in the NIRS equations for pH with the loss of spectral information on scanning or analysing samples of minced meat; in this study, scanning intact pork allows the maintenance of muscle structure and as a consequence the light scattering properties[17]. However, the results obtained only allow determination coefficients of cross validation between 0.2 and 0.5. According to Berzaghi et al.[21] the low variation of pH value measurements (between 5.2 and 6.6) may have reduced NIR predictability in pork meat samples.

Our results are according with those obtained for beef with an at-line Mid IR instrument[19]. In this sense, the use of this innocuous, robust and low cost portable

handheld NIR instrument to predict shelf life of pork meat on-site NIR instruments (range 1100-2500nm) could allow the establishment in real time (for example at supermarket level) of controls of the state of meat preservation with a qualitative response regarding the conservation state of the sample (acceptable or not for consumption).

Another alternative that may be useful for establishing a real time control of the state of meat preservation is related with a qualitative response regarding the conservation state of the sample (acceptable or not for consumption) and the legislation[18]. Table 2 presents aerobic *Mesophilous* microorganism data and the NIR statistics obtained for the best predicting model developed. The values for coefficient of determination for cross validation were close to 0.33 and 0.40 for gas A and B respectively. The best statistics were obtained when grouping data from gas A and B(r^2= 0.66). The standard error of cross validation was near to five times less than standard deviations of calibration population. The big residual errors were identified as being related with samples included in the limit between allowed and forbidden, from 6.0 to 7.0 log cfu/gr aerobic *Mesophilous* microorganisms.

The r^2 and SECV values obtained for qualitative and for quantitative models of mesophilic bacteria are acceptable as a legislative parameter to establish the limit of acceptability. In this sense, NIR spectroscopy *on-site* developed methodology could be an alternative to determine shelf life extension on commercial trays of pork meat slices exhibited in a supermarket.

The next step was to evaluate the alternative of developing NIRS calibrations to estimate the microbiological parameters by using the spectra collected on sample before opening the package as an alternative to use the predict results directly on trays displayed on commercial exhibitors in supermarkets. The results obtained with all pre-treatment as detailed in the Material and Methods section and with both first and second derivative were not satisfactory, the best statistics for r^2 were 0.037-0.136 for aerobic *Mesophilous* microorganisms and Gas A and B respectively. An interpretation of these results could be that when collecting the meat spectra on their packaging, the head space between the film and pork meat surface leads to lower sensitivity of the NIR signal. It is not easy to establish adequately the contact between the sample and the NIR sensor and the spectral information has a lot of interferences which are difficult to avoid for the collection of high-quality spectra. High-quality spectra are critical to the subsequent development of models and prediction calibrations[21]. Furthermore, these poor results are related with the difficulty of scanning intact meat samples[17].

CONCLUSIONS

The use of the portable NIR instrument evaluated in this work for the prediction of microbiological parameters in packaged sliced pork meat with different modified atmosphere was shown that it could be a novelty analytical sensor to establish the meat conservation state of pork meat samples displayed on commercial exhibitors in supermarkets, as screening tool to select samples acceptable or not for consumption. Results have shown that there are not influence of MAP on calibration statistics. However, further work remains to be done to develop robust NIRS models increasing precision and accuracy (around 0.3 for reference method). Moreover, other on-site NIR

instruments with different or wider wavelength ranges must be evaluated before implementing the methodology in routine analysis.

Acknowledgements

This research work was supported by the Asturias Regional Project PCTI- IE07-132 and PCTI-IB08-151 for portable NIRS instrument acquisition.

REFERENCES

1. P. Díaz, M. D. Garrido, S. Bañón, Meat Sci. 84, 538 (2010).
2. A. Nopwinyuwong, , S. Trevanich, P.Suppakula,, Talanta 81, 1126 (2010).
3. F. Pérez-Alonso, S.P.Auburg, O. Rodríguez, J. Barros-Velázquez, Eur. Food Res. Technol. 218, 313 (2004).
4. E. Chouliara, A. Badeka, I. Savvaidis, M.G. Kontominas, Eur. Food Res. Tech. 226 (4), 877 (2007).
5. Y. Liu, , B.G .Lyon, W.R Windham, C.E. Lyon, E.M Savage, Poultry Science, 83, 1467-1474 (2004)
6. G.H Geesink., F.H. Schreutelkamp, R. Frankhuizen, H.W. Vedder, N.M Faber, R.W. Kranen, Meat science, 65, 661-668 (2003)
7. M. T. Sánchez, M. D. Pérez-Marín, K. Flores-Rojas, J. E Guerrero, A. Garrido-Varo, Talanta, 78, 530–536 (2009).
8. N. Prieto, R. Roehe, P. Lavín, G. Batten, S. Andrés, Meat Sci., 83, 175 (2009)
9. G. Downey, K.I. Hildrum, Analysis of meats. In L. Al-Amoodi, R. Craig, J. Workman, & J. Reeves, III (Eds.), *Near-infrared in agriculture* Madison pp. 599-632. (2004).
10. J. S. Shenk, M. O. Westerhaus, Crop Sci. 31: 1548 (1991).
11. R.J. Barnes, M.S. Dhanoa, S.J. Lister, Appl. Spectrosc. 43 (5), 772 (1989).
12. R.J. Barnes, M.S.Dhanoa, S.J. Lister, J. Near Infrared Spec. 1(3), 185 (1993).
13. WinISI II software v.1.5, Infrasoft International, Port Matilda, PA, USA, (2000).
14. N. Prieto, S. Andrés, F.J. Giráldez, A.R. Mantecón, P. Lavín,), Meat Sci. 79, 198 (2008)
15. B. G. Osborne, T. Fearn, Near infrared spectroscopy in food analysis. Longman Scientific and Technical. pp. 36 (1986)

16. P.D. Warriss, Handbook of Meat Science. The chemical composition and Structure of Meat (2nd ed.). Cambridge University Press, p.27 (2010)
17. D. Cozzolino, I. Murray, J. Near Infrared Spec., 10, 37 (2002).
18. European Union. Regulation (EC) No.1441/2007. Off. J. Eur. Comm. L 322/12-29 (2007).
19. A.A. Argyri, E.Z. Panagou, P.A. Tarantilis, M. Polysiou, G. J.E. Nychas, Sensors and Actuators B: Chemical, 145, 146 (2010)
20. M.S. Ammor, A. Argyri, G.J. E Nychas, Meat Sci, 81, 507 (2009).
21. P. Berzaghi, A. Dalla Zotte, L.M. Jansson, , I. Angdrighetto, Poultry Sci, 84, 128 (2005).
22. ISO/PDTS 19036. Microbiology of food and animal feeding stuffs- Guide on estimation of measurements uncertainty for qualitative determinations. (2005).

Legends of Figures

Figure 1.-NIR mean spectra and Second derivatives of pork meat samples acceptable (A) and No-Acceptable (NA) for consumption and packed in modified atmosphere with gas A. Gas A: Freshline™ 3MIX (30/ 40/ 30: $O_2/CO_2/N_2$);; Gas B: and Freshline™ 3MIX 20/5 (5/20/75: $O_2/CO_2/N_2$). A= aerobic *Mesophilous* microorganisms <6.699 log cfu/g; NA= aerobic *Mesophilous* microorganisms >6.699 log cfu/g

Figure 2.-First derivatives of pork meat samples acceptable (A) and No-Acceptable (NA) for comsumption and packed in modified atmosphere with gas A and gas B. Gas A: Freshline™ 3MIX (30/ 40/ 30: $O_2/CO_2/N_2$);; Gas B: and Freshline™ 3MIX 20/5 (5/20/75: $O_2/CO_2/N_2$). A= aerobic *Mesophilous* microorganisms <6.699 log cfu/g; NA= aerobic *Mesophilous* microorganisms >6.699 log cfu/g

Figure 3.- Evolution of aerobic *Mesophilous* microorganisms, lactic acid bacteria and enterobacteriacea under different atmospheres packaging. Gas A: Freshline™ 3MIX (30/ 40/ 30: $O_2/CO_2/N_2$);; Gas B: and Freshline™ 3MIX 20/5 (5/20/75: $O_2/CO_2/N_2$).

Figure 4.- Comparison of predicted vs. reference data for optimised calibration of aerobic *Mesophilous* microorganisms with gás A and B. Gas A: Freshline™ 3MIX (30/ 40/ 30: $O_2/CO_2/N_2$);; Gas B: and Freshline™ 3MIX 20/5 (5/20/75: $O_2/CO_2/N_2$)

Table 1.- Calibration statistical parameters for quantitative prediction of microbiological parameters and pH of intact pork samples packed with different controlled atmospheres with NIR spectroscopy

Parameter	Gas	Pre and Math treatment	CV	SEC Log ufc/g	R^2	SECV Log ufc/g	r^2	RER	RPD
Aerobic mesophiles log cfu/g	A	SNVD 1,5,5,1	0.417	0.949	0.719	1.063	0.654	6.33	1.68
	B	SNVD 1,5,5,1	0.246	0.972	0.619	1.079	0,536	8.80	1.45
	A+B	MSC 2,4,4,1	0.197	0.763	0.641	0.817	0.596	9.35	1.56
Enterobacteriaceae log cfu/g	A	SNVD 0,4,4,1	0.497	1.074	0.340	1.198	0.191	4.41	1.10
	B	MSC 2,4,4,1	0.417	1.010	0.492	1.153	0.343	4.53	1.23
	A+B	MSC 2,4,4,1	0.140	0.263	0.562	0.342	0.420	6.97	1.16
Lactic acid bacteria log cfu/g	A	SNVD 1,5,5,1	0.370	1.137	0.674	1.256	0.604	5.81	1.58
	B	MSC 0,4,4,1	0.331	1.058	0.653	1.296	0.485	5.65	1.39
	A+B	MSC 2,4,4,1	0.260	0.950	0.596	0.982	0.571	9.13	1.52
pH	A	SNVD 1,5,5,1	0.024	0.088	0.583	0.100	0.472	8.90	1.36
	B	SNVD 1,5,5,1	0.022	0.103	0.392	0.117	0.206	6.50	1.08
	A+B	MSC 2,4,4,1	0.010	0.035	0.555	0.039	0.467	8.09	1.35

Gas A: Freshline™ 3MIX (30/ 40/ 30: $O_2/CO_2/N_2$);; Gas B: and Freshline™ 3MIX 20/5 (5/20/75: $O_2/CO_2/N_2$); cfu/g: colony forming units per gram; CV: Coefficient of variation (SD/mean); SEC: standard error of calibration; R^2 : coefficient of determination; SECV: standard error of cross validation; r^2: coefficient of determination for cross validation; SNVD: Standard Normal Variate and Detrend; MSC: Multiplicative scatter correction; RER: Range/ SECV; RPD: Standard deviation/SECV

Table 2.- Calibration statistical parameters for qualitative prediction of aerobic mesophiles of intact pork samples packed with different controlled atmospheres with NIR spectroscopy

Parameter log cfu/g	Gas	Pre and Math treatment	CV	SEC Log ufc/g	R^2	SECV Log ufc/g	r^2	RER	RPD
Aerobic mesophiles	A	MSC 1,5,5,1	0.337	0.367	0.465	0.414	0.328	1.21	1.21
	B	SNVD 1,5,5,1	0.344	0.345	0.523	0.388	0.404	2.57	1.28
	A+B	MSC 1,5,5,1	0.678	0.541	0.703	0.582	0.657	4.88	1.70

cfu/g: colony forming units per gram; Gas A: Freshline™ 3MIX (30/ 40/ 30: $O_2/CO_2/N_2$); Gas B: and Freshline™ 3MIX 20/5 (5/20/75: $O_2/CO_2/N_2$); cfu/g: colony forming units per gram; CV: Coefficient of variation (SD/mean); SEC: standard error of calibration; R^2 : coefficient of determination; SECV: standard error of cross validation; r^2: coefficient of determination for cross validation; SNVD: Standard Normal Variate and Detrend; MSC: Multiplicative scatter correction; RER: Range/ SECV; RPD: Standard deviation/SECV

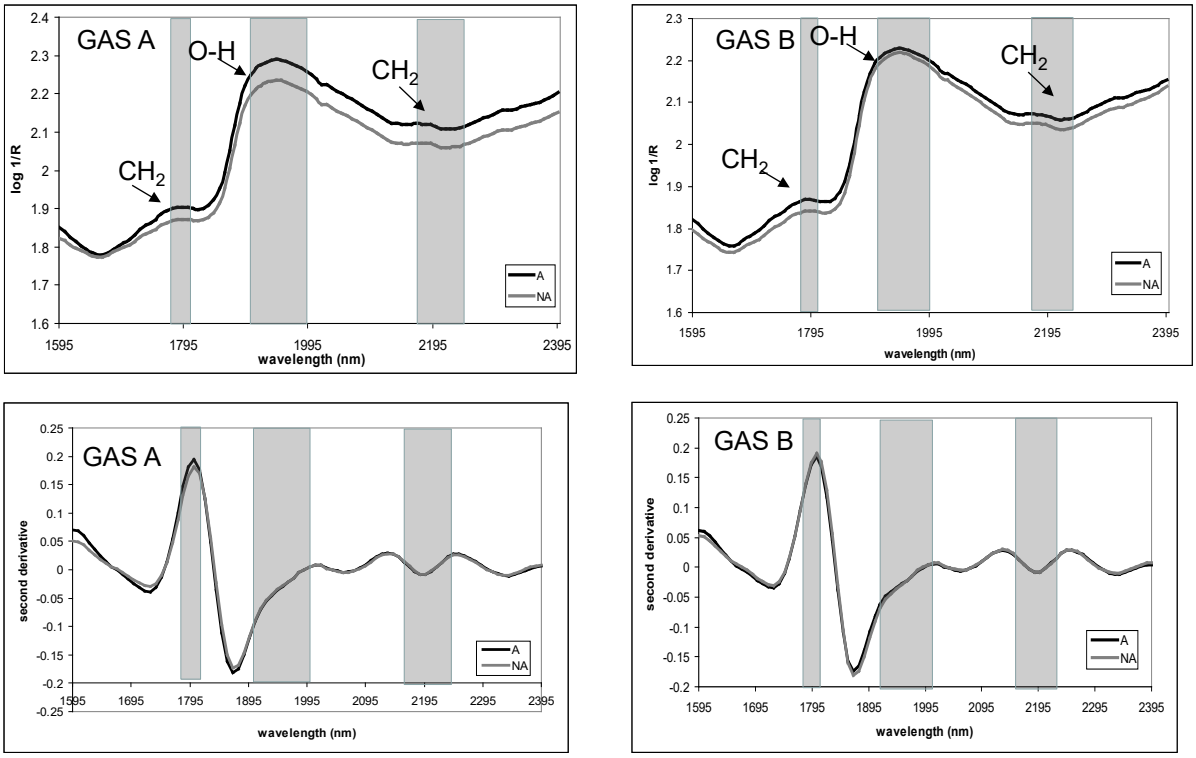
GAS A
O-H
CH2
CH2
log 1/R
wavelength (nm)
A
NA
GAS B
O-H
CH2
CH2
log 1/R
wavelength (nm)
A
NA
GAS A
second derivative
wavelength (nm)
A
NA
GAS B
second derivative
wavelength (nm)
A
NA

FIGURE 1

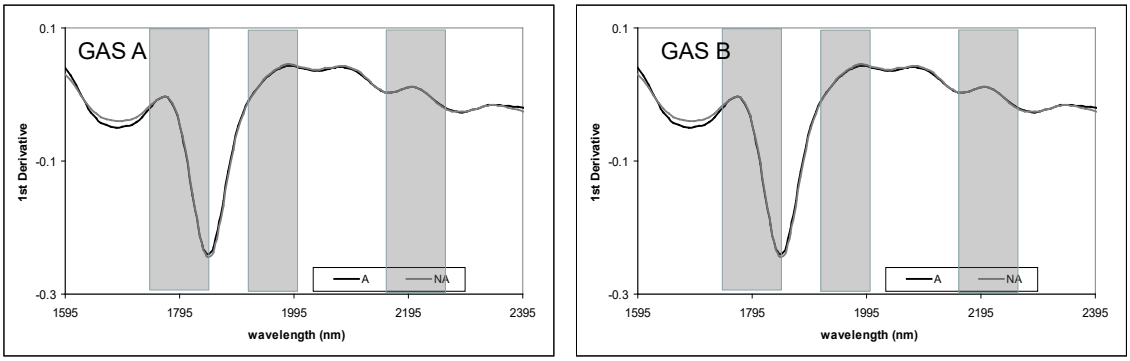
GAS A
GAS B
1st Derivative
0.1
-0.1
-0.3
1595
1795
1995
2195
2395
wavelength (nm)
A
NA

FIGURE 2

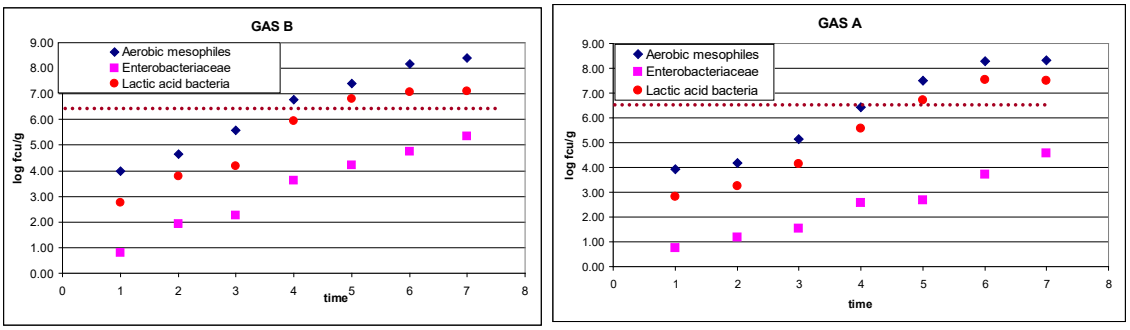
GAS B
Aerobic mesophiles
Enterobacteriaceae
Lactic acid bacteria
log fcu/g
time
GAS A
Aerobic mesophiles
Enterobacteriaceae
Lactic acid bacteria
log fcu/g
time

FIGURE 3

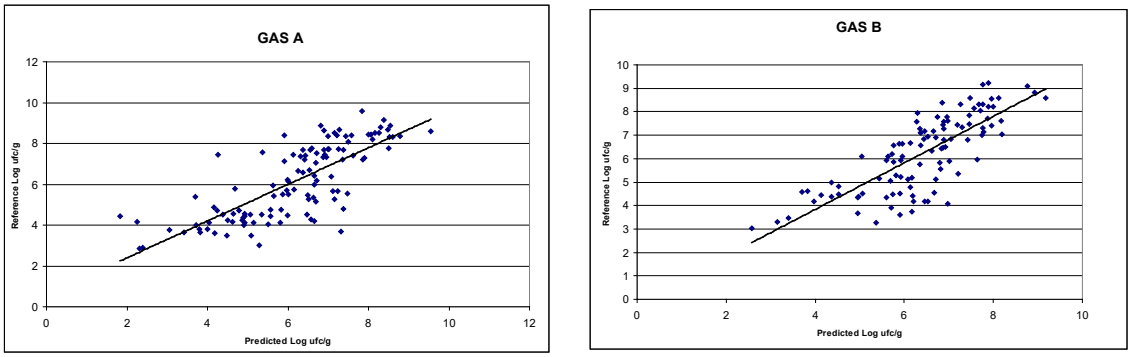
GAS A
Reference Log ufc/g
Predicted Log ufc/g
0
2
4
6
8
10
12
GAS B
Reference Log ufc/g
Predicted Log ufc/g
0
1
2
3
4
5
6
7
8
9
10

FIGURE 4