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RAPID DETERMINATION OF LIPID OXIDATION IN MECHANICALLY DEBONED TURKEY MEAT BY AUTOFLUORESCENCE SPECTROSCOPY.

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Background:
Lipid oxidation is a major cause of muscle food deterioration, and many efforts have been made to develop rapid methods to detect and quantify lipid oxidation in food systems. A non-destructive technique would have important advantages, since rancidity could be monitored on-line during processing and the extent of oxidation could be measured without being affected by any extraction process. Autofluorescence has been demonstrated to be an excellent indicator of lipid oxidation in biological materials (Melton, 1983), and should be likely to succeed as non-destructive. A variety of oxidation products are formed during oxidation processes and some of these are autofluorescent, formed from reactions of oxidising fatty acids or lipid oxidation breakdown products (aldehydes, malondialdehyde, hydroperoxides) with compounds containing primary amino groups (proteins, amino acids, DNA, phospholipids) (Kikugawa and Beppu, 1987). Autofluorescence has so far been used mainly on extracts of lipids or proteins, and the intensity of emission usually measured at one single wavelength. Hasegawa et al. (1992; 1993), however, measured fluorescence directly on a dried fish model system and freeze-dried pork and egg yolk and obtained promising results with regard to rancidity. Except for these reports, we have not found any attempts to utilise the fluorescent properties of oxidised foods to develop a rapid and non-destructive method for assessment of oxidative deterioration.

Mechanically deboned turkey meat (MDTM) was chosen as model material because of high concentrations of polyunsaturated fatty acids (PUFA). MDTM is widely used in chopped emulsion poultry products. The quality change that occurs in frozen stored MDTM as raw material for further production is due to rapid development of rancidity. Cell disruption and excessive aeration during the process as well as extraction of heme and lipids from bone marrow are the main reasons for high susceptibility of MDTM to oxidative deterioration.

Objective:
The aim of this study was to investigate the feasibility of solid sample autofluorescence spectroscopy as a method to measure the extent of lipid oxidation in MDTM.

Materials and Methods:
MDTM was obtained from a commercial processing plant. The meat was processed trough Beehive deboner from hand-boned turkey's skeleton including backs with natural portion of skin or neck without skin or blend of these. Chilled MDTM collected from 11 different batches were vacuum packed in plastic bags or in plastic boxes with air access and then stored at -25 °C. The different wrapping were chosen in order to span the range of lipid oxidation.

Proximate composition, including moisture, total fat, protein and connective tissue protein was determined on duplicates from MDTM according to AOAC methods (1990). The extent of lipid oxidation was assessed by the 2-thiobarbituric acid (TBA) assay. Frozen MDTM samples were thawed at 4°C prior to analysis. The thiobarbituric acid reactive substances (TBARS) were determined in duplicate by the extraction procedure according to Sørensen and Storgaard Jørgensen (1996). The TBARS values were expressed as mg of malondialdehyde per kilogram of meat. Samples were collected and examined during one year frozen storage.

Autofluorescence measurements were performed simultaneously with TBA assay on the same samples. A total of 49 measurements were carried out during storage period (8 runs). Fluorescence emission spectra were measured by an optical bench system. The excitation wavelengths 380 nm were generated by a 300 W Xenon light source, a heat absorbing filter and 10 nm bandwidth interference filters (d=50 mm) mounted in a filter wheel. The light was directed onto samples at an angle of 45°. The samples (about 200 g) were placed into specially designed sample cuvettes which exposed a flat circular surface (d=50 mm) for measurement. A sharp cut-off filter was positioned in front of the detector to prevent reflected excitation light from overlapping the autofluorescence light. The detector used was a 512*512 charge coupled device - *Princeton* CCD camera. It was cooled to -40°C generating a low dark charge. En *Acton SP-150* imaging spectrograph was connected to the CCD. Autofluorescence was measured in the range 400-640 nm. Samples were illuminated throughout exposure (Δt = 5 sec). Temperature of samples was 16-20 °C. The black painted laboratory was 18 °C and had a minimum of straylight.

Statistical analysis
The emission spectra from each sample were used to predict chemical reference measurements of TBARS. The multivariate calibration technique partial least squares regression (PLS) (Martens and Næs, 1989) was chosen, since this method has been reported to successfully resolve autofluorescence spectra from complex mixtures and turbid samples. The multivariate correlation coefficient (R) and the prediction error expressed as root mean square error of cross validation (RMSECV) were used to evaluate the models.

Results and Discussion:

Chemical measurements

The different types of MDTM spanned well the range of fat (6.7-17.6), moisture (65.6-74.6), protein (16.3-17.6) and connective tissue (0.9-1.4) contents in the samples. Samples with high amount of lipid contained less moisture. TBARS values ranged from 0.07 to 5.1 mg malondialdehyde/kg meat. The mean standard deviation of the parallels was 0.05, but we noticed an increase in standard deviation with increasing TBARS values indicating greater uncertainty for these.

Fluorescence measurements

Typical emission spectrum from fresh and rancid MDTM for 380 nm excitation wavelength is shown in Figure 1. The sharp peak at 475 nm was probably related to collagen and had also been observed in bovine meat (Wold, 1999). Storage led to increased fluorescence over almost the entire measured range. The multivariate analysis indicated that this fluorescence was composed by two peaks, one at about 456 nm and the other around 525 nm. This corresponded with the findings of Kikugawa and Beppu (1987) and Hasegawa et al. (1992;1993)

The regression models showed that there was a clear relationship between fluorescence and TBARS values (Figure 2). It was quite clear, however, that the relation was non-linear. Since PLS models linear relationships, all regression were made against log (TBARS). Prediction results were then transformed back to TBARS values. First set with all samples (n=49) covering an extensive range of TBARS values (0.07 and 5.1) resulted in a model of 4 PLS factors with correlation values 0.93 and 0.95 for log(TBARS) and the converted TBARS, respectively. The prediction error RMSECV was 0.385. Correlation values were lower (0.90 and 0.80, respectively) for a reduced sample set (n=44) where the very rancid samples were omitted. This set spanned a realistic commercial range of TBARS values (0.07-1.7). Note, however, that the RMSECV of this 4-factor model was considerably lower (0.283). This was because the models were more precise in the low-level area, probably because fresh samples had very similar spectra, whereas spectra from severely oxidised samples differed more, most likely due to variations in oxidative processes.

Conclusion:

Solid sample autofluorescence spectroscopy appeared to be well suited for non-destructive determination of lipid oxidation level in MDTM. Samples of low TBARS values were better predicted than of high TBARS values. Fluorescence could apparently detect early stages of lipid oxidation. Multivariate analysis is necessary for optimal regression. The results suggest that an on-line application for detection of lipid oxidation is possible.

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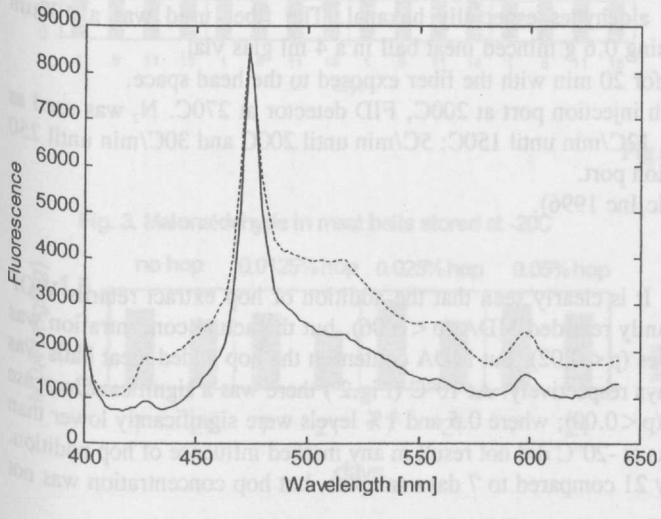

Figure 1 Fluorescence spectra from fresh (____) and lipid oxidised (-----) MDTM

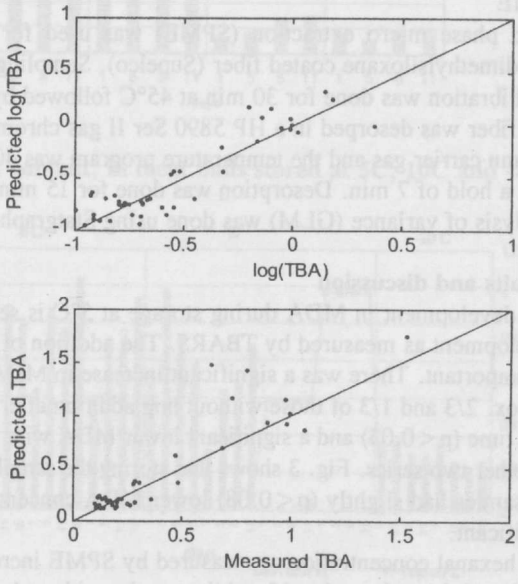

Figure 2 Predicted versus measured plots