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Determination of Free Fatty Acids (FFA), Peroxide Value (PV), and p-Anisidine Value (p-AV) of Marine Oil using the Fourier Transform Near Infrared (FT-NIR) Spectroscopy

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ABSTRACT Marine oils have Omega-3 polyunsaturated fatty acids (PUFA) that will oxidize rapidly during extraction, refining, bleaching, deodorizing, and storage when unfavorable conditions exist. When oil oxidation occurs, the peroxide value (PV) and p-anisidine value (p-AV) increase. Improper refining and deodorization conditions can lead to higher free fatty acid (FFA) contents in the processed marine oil. The wet chemistry methods are usually time-consuming, skilled-labor intensive, and require significant amounts of chemical reagents for performing the analyses. An inclusion of a rapid determination technique for FFA, PV, and p-AV is considered vital for the oil processing industries. The objective of this study was to develop prediction models for FFA, PV, and p-AV of marine oil using FT-NIR spectroscopy. Standard wet chemistry results were used as reference points for developing FT-NIR models for predicting the FFA, PV, and p-AV for bleached and deodorized marine oil samples. The FT-NIR prediction models were developed by analyzing marine oil samples on an FT-NIR spectrometer with the scans taken between 4,500 and 12,000 cm^{-1} . Coefficients of determination (R^2) of 92.83, 97.00, and 93.57 for FFA, PV, and p-AV,

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respectively, were attained from the spectroscopic analysis. The Root Mean Square Error for Cross Validation (RMSECV) values were 0.0186, 0.612, and 0.662 for FFA, PV, and p-AV, respectively. In this study, it was demonstrated that the FT-NIR spectroscopy can be used for predicting the FFA, PV, and p-AV of the marine oil.

Keywords Peroxide Value, para-Anisidine Value, Free Fatty Acid, Fourier Transform near Infrared Spectrometer

INTRODUCTION The consumer demand for oils containing Omega-3 fatty acids is growing rapidly, due to the purported health benefits. Three types of common Omega-3 fatty acids are: alpha-linolenic acid (ALA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). Some marine oils are rich in the EPA and DHA whereas some plant oils have high amounts of ALAs (DrAxe, 2017). The oxidative quality of oils is tested mainly by measuring the FFA, PV (primary oxidation products), and p-AV (secondary oxidation products). The FFA value is a measure of the free fatty acids that can be released due to a break in the ester linkage connecting the fatty acids to the glycerol “backbone” of the triacylglycerol (oil) molecule. The PV indicates the primary oxidation products (mainly peroxides) that can be formed from the reaction of oxygen with the double bond of a fatty acid. The p-AV is the value of secondary oxidation products (mainly aldehydes and ketones) that can be formed by reaction of oxygen with the fatty acid chains in the oil.

As the oil containing Omega-3 fatty acids are prone to oxidation, they are typically processed in an inert environment and the resulting processed oil is stored in light inhibiting containers/packaging. However, minimizing the oxidation during processing or testing of oils is a challenge to the oil processing industries. The standard wet chemistry methods for FFA, PV, and p-AV are time consuming and require trained people to perform the analyses. Furthermore, these methods also require various chemical reagents. Therefore, safe handling of chemicals is required. The samples are degraded during analysis. A fast and reliable method to measure FFA, PV, and p-AV will add significant efficiency to analysis of Omega-3 oils.

FT-NIR spectroscopy has been used for wide range of applications, including investigation of authenticity of edible oils and fats (Yang et al., 2004), adulteration of sesame oil (Luo et al., 2012), quantification of fatty acids and their ethyl esters in fish oil (Azizian et al., 2010), determination of methyl ester contents in biodiesel blends (Oliveira et al., 2006), and adulteration of diesel/biodiesel blends by vegetable oil (Oliveira et al. 2007). The FT-NIR spectroscopy can be used a rapid method for quantifying the fatty acids in vegetable based fats and oils (Azizian and Kramer, 2005).

The objective of the current study was to develop prediction models for FFA, PV, and p-AV for marine oil using the FT-NIR spectroscopy.

MATERIALS AND METHODS A total of 58 marine oil samples, that were high in EPA and DHA, were analyzed for the FFA and PV. Twenty nine samples were analyzed for p-AV. These samples were provided by one of the clients of POS Biosciences, Saskatoon, SK. The standard methods from American Oil Chemists' Society (AOCS) were used for the FFA (Method: AOCS-Ca 5a-40) and PV (Method: AOCS Cd 8b-90) analyses in the lab. The International Organization for Standardization (ISO) method was used for the p-AV (Method: ISO/CD 6885.2) analysis. The FFA, PV, and p-AV results were used as references for the development of the FT-NIR prediction models for calibration and validation purposes. The leave-one-out cross validation method was used for validation purposes. Samples were scanned using the FT-NIR Multi-Purpose Analyzer (MPA) (Bruker Optics, Ettlingen, Germany), shown in Figure 1, for acquiring spectra from 4,500 to 12,000 cm^{-1} . The background scans were taken prior to scanning samples. The wet chemistry analyses and FT-NIR scans were performed the same day for each sample for PV and FFA analysis. As the same day p-AV analyses could not always be completed in one day, samples were held in frozen storage for p-AV analysis and also for the FT-NIR scan for p-AV correlation. For such samples, both

the wet chemistry (p-AV) and FT-NIR scans were completed within two days after collecting the samples. The OPUS 5.5 spectroscopy (Bruker Optics, Ettlingen, Germany) software was used for developing the prediction models using the partial least square regression (PLSR) with the combination of few data preprocessing methods such as first derivatives, second derivatives, multiplicative scatter correction, and mean centering.



Figure 1. The FT-NIR Multi-Purpose Analyzer.

RESULTS AND DISCUSSION The FT-NIR spectra of the marine oil samples are given in Figure 2. Three sample scans were shown in the spectral profile.

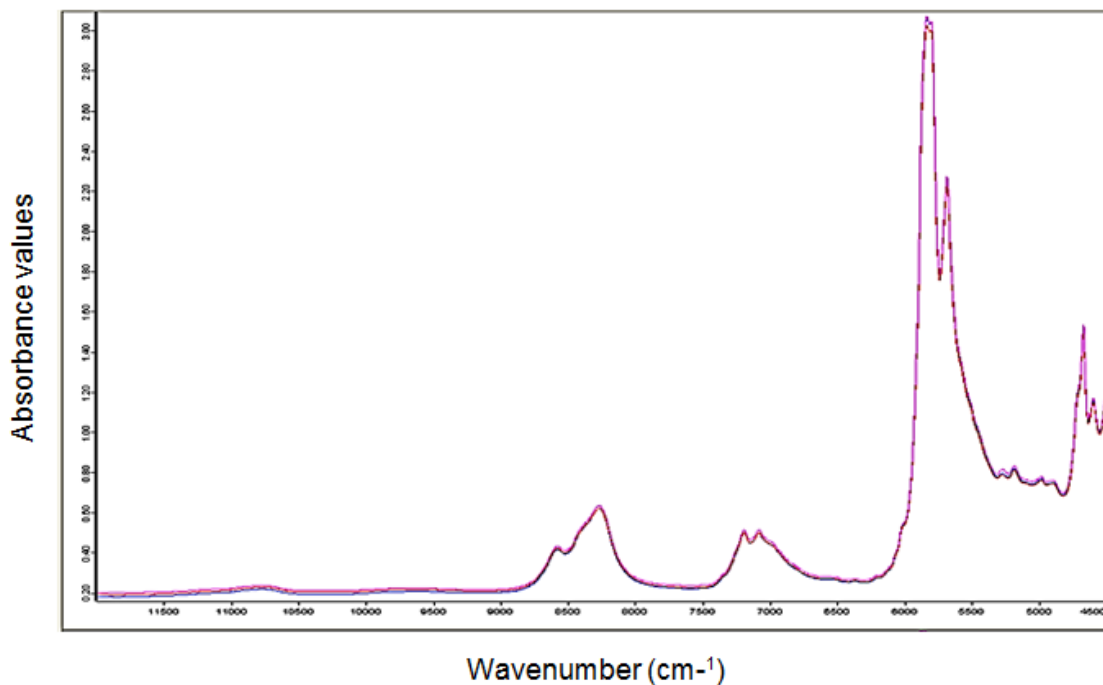


Figure 2. The FT-NIR spectra of the marine oil samples.

The R^2 of the FFA was 92.83 with an RMSECV of 0.0186 (Figures 3 and 4). All 10 partial least square ranks were used for developing the model. First derivatives method, multiplicative scatter correction, and mean centering were used for preprocessing of the spectra with 17 smoothing points. Spectral regions of 4,497.5 to 5,454.0 cm^{-1} and 6,098.2 to 11,995.8 cm^{-1} were used for calibration and validation purposes of the FFA prediction model.

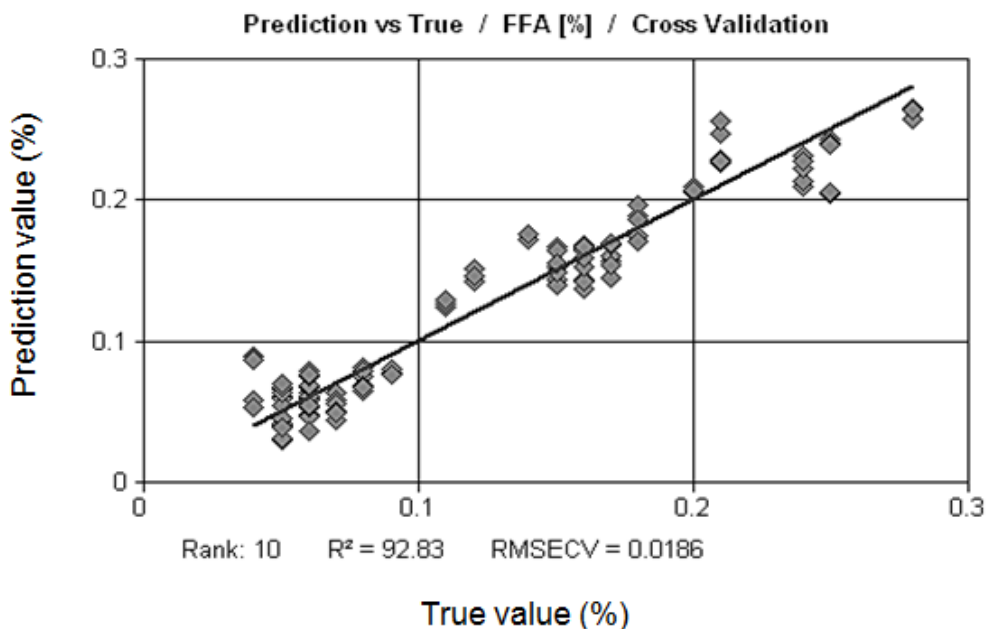


Figure 3. Prediction vs. true values for the FFA values of marine oil samples.

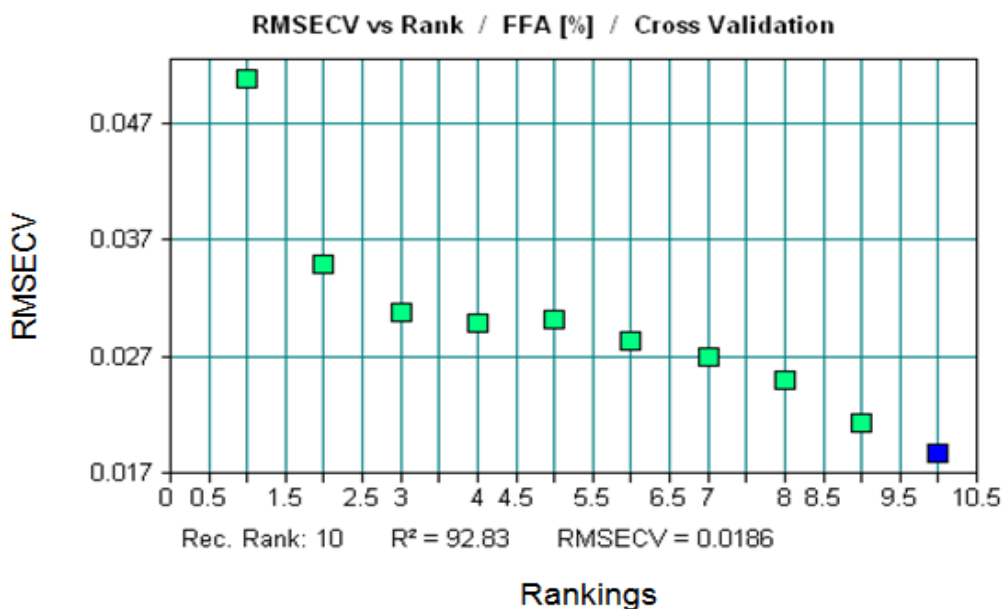


Figure 4. The RMSECV for the PLS rankings of the prediction model for FFA.

The PV model produced an R^2 of 97.0 with and RMSECV of 0.612 (Figures 5 and 6). The first 9 PLS ranks were used for developing the model. Second derivatives method and mean centering were used for preprocessing of the spectra with 17 smoothing points. Spectral regions of 4,593.9 to 5,454.0 cm^{-1} were used for calibration and validation purposes of the PV prediction model.

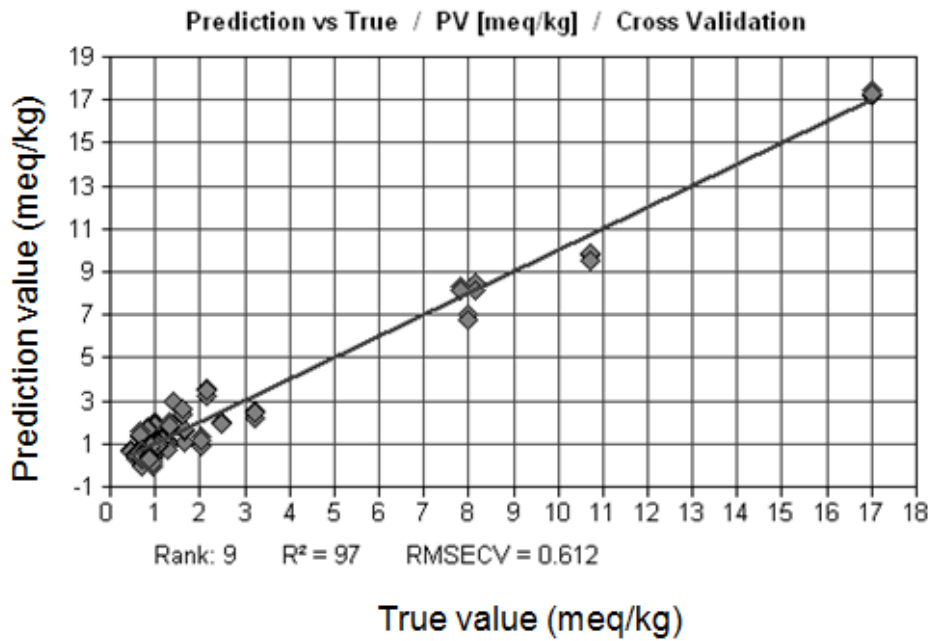


Figure 5. Prediction vs. true values of the PV values of marine oil samples.

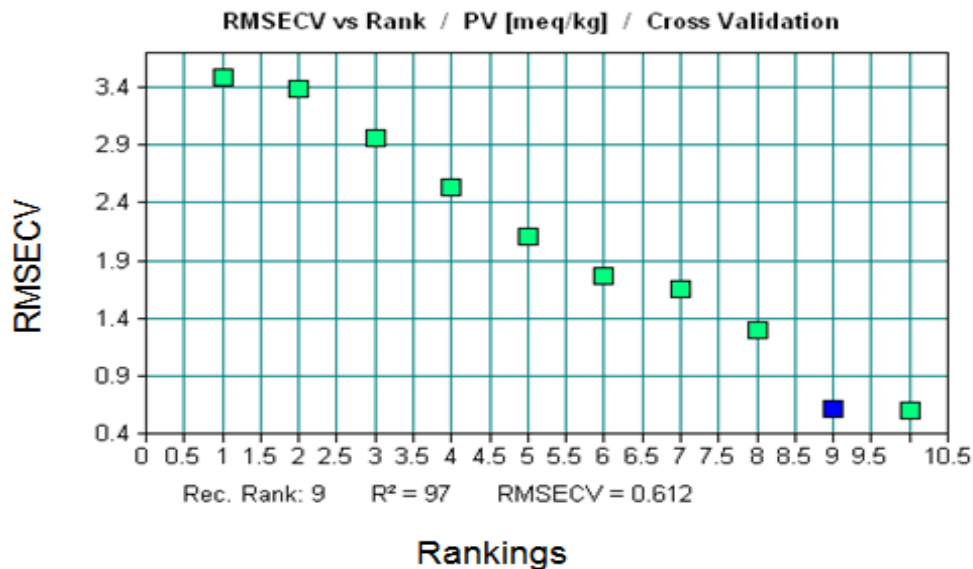


Figure 6. The RMSECV for the rankings of the prediction model for PV.

The p-AV model resulted in an R^2 value of 93.57 with a RMSECV of 0.662 (Figures 7 and 8). First 10 PLS ranks were used for developing the model. Multiplicative scatter correction with mean centering was used for the preprocessing of the NIR spectra. Spectral regions of 5,022.0 to 5,450.2 cm^{-1} and 6,098.2 to 9,048.9 cm^{-1} were used for calibration and validation purposes of the PV prediction model.

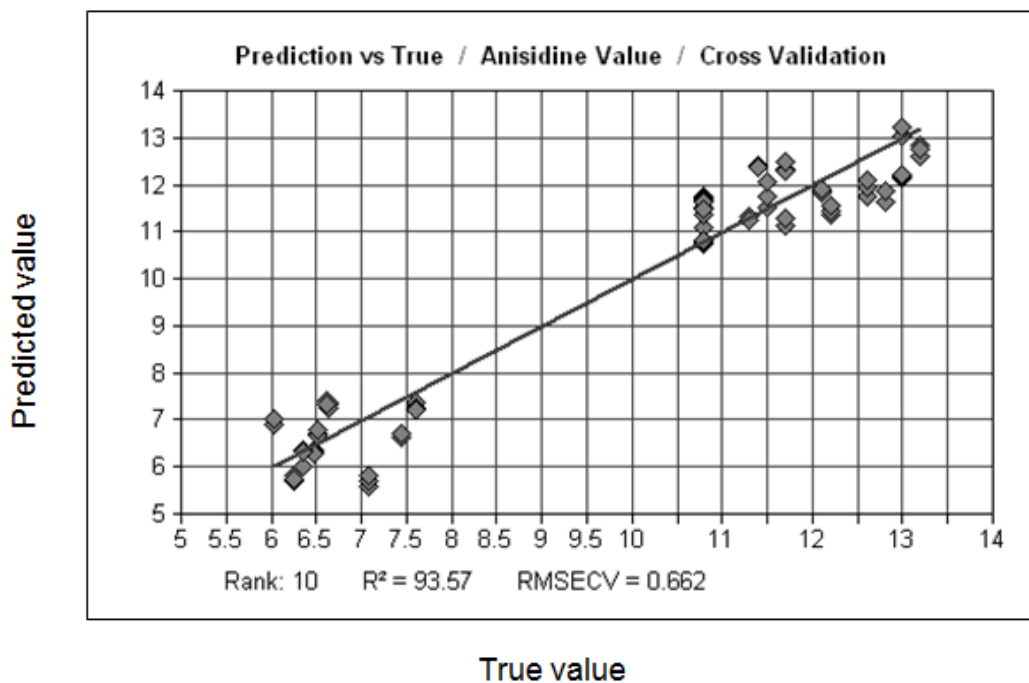


Figure 7. Prediction vs. true values of the p-AV values of marine oil samples.

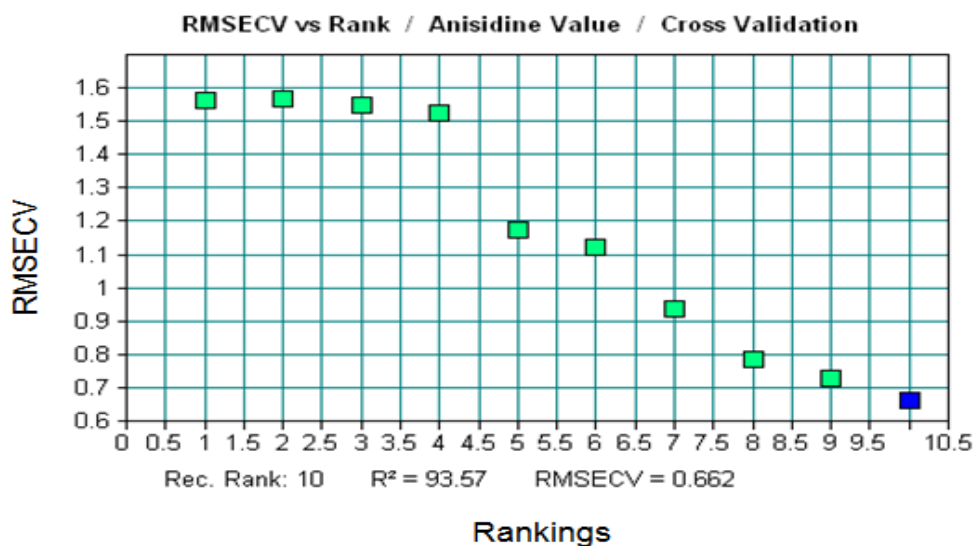


Figure 8. The RMSECV for the rankings of the prediction model for p-AV.

Azizian and Kramer (2005) have used the FT-NIR spectroscopy for quantification of fatty acids in fats and edible oils previously. They reported that the method has potential to be used a rapid measurement tool for fat products. In our study, all of the prediction models had a coefficient of determination (R^2) value of >92 with the RMSECV of <0.662. The results in this study demonstrate that FT-NIR spectroscopy can be used as a quick-measurement tool for the FFA, PV, and p-AV in marine oils.

CONCLUSION It was demonstrated from this study that the FT-NIR spectroscopy could be used as a quick analytical tool to measure the FFA, PV, and p-AV in marine oils containing Omega-3 fatty acids. This study has shown a strong correlation between the wet chemistry methods and the FT-NIR model developed, including calibration, validation, and prediction of unknown samples, in depth for FFA, PV, and p-AV for marine oils. The FT-NIR methods could also be used as an in-line quality monitoring tool for the marine oil processing industry.

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