JOURNAL OF UNDERGRADUATE RESEARCH

Application of VIS-NIR Hyperspectral Imaging for the Detection and Quantification of MPs in Simulant Shellfish

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Abstract

As the world has become increasingly reliant on single-use plastics, plastic litter has increased exponentially, especially in marine ecosystems. One type of plastic litter affecting marine life is microplastics, which are plastics under 5mm in diameter. Microplastics are consumed by marine animals, which are then consumed by people. Study of the environmental and human health effects of these microplastics has proven difficult, in part due to difficulty detecting and quantifying microplastics. This study aims to detect microplastics in shellfish by utilizing the nondestructive method of hyperspectral imaging as an indicator of both marine ecosystem health and food quality. Gelatin models of shellfish were injected with a known concentration of microplastics (polyvinyl chloride (PVC) and polystyrene (PS), specifically). The hyperspectral camera then took 3-dimensional images of the models, with the wavelength dimension ranging from 400 nm to 1000 nm. Next, an algorithm called Partial Least Squares Regression Analysis was developed to analyze the relationship between the wavelengths and the amount of plastic in the sample. Preliminary results have shown slight variation in reflectance spectra between models spiked with microplastics and control models past the 950 nm range. Therefore, hyperspectral imaging might be a viable tool to detect and quantify microplastics in shellfish, but the range of the camera used in this study is a limitation.

Keywords: Hyperspectral Imaging, Microplastics, Polystyrene, Polyvinyl Chloride, Shellfish

Introduction

Microplastics (MPs) are defined as any plastics between 1μm and 5mm in diameter (Frias & Nash, 2019) that are insoluble in water. Due to their insolubility and small size, these plastics pose a serious threat to marine ecosystems. MPs can be ingested by marine organisms and then transferred via the food chain (Wright et al., 2013). This study specifically focuses on MPs ingested by shellfish for their filtration properties, their use in indication of environmental health, and their potential to be consumed by humans.

Shellfish are categorized as filter feeders. Filter feeders have been studied as potential indicator species of marine environment health (Fossi et al., 2014) as well as natural filters for

excess nutrients (Rose et al., 2015) and microorganisms, such as viruses (Maalouf et al., 2011). It has also been proposed that filter feeders and shellfish could be indicators and filters of microplastics in marine ecosystems (Su et al., 2018).

In addition to their role in environmental studies, filter feeders are also important to the human diet Potential health implications of human MP consumption (Campanale et al., 2020), make it important to be able to detect these microplastics. Not only this, but it is important to also be able to detect microplastics in a nondestructive manner so that shellfish meant for human consumption can be checked for microplastics without ruining the food. Therefore, this study proposes to apply hyperspectral imaging to detect microplastics in shellfish. Hyperspectral imaging (HSI) is a nondestructive imaging technology that generates a 3-dimensional hypercube, which is the 2-dimensional spatial image with the third dimension being the spectral information, thus a spectrum is generated at each image pixel (Sun, 2010). HSI takes advantage of the chemical interactions within samples' spectral data in combination with the spatial data so that chemical composition and location can be determined.

As the first step in applying HSI in the identification of MPs in shellfish, this study uses gelatin models to represent shellfish, due to having similar physical characteristics. Additionally, using model shellfish allows for the samples' composition to be controlled, thereby creating a consistent model with a known composition. This also is a less expensive and more practical method than buying fresh, live shellfish samples. If successful detection of MPs in gelatin models could be achieved using the nondestructive spectroscopic method of HSI, the next step would be to replicate the process with real shellfish samples. Some changes in the methodology may be necessary, such as accounting for differences in color, texture, or density of the shellfish samples. However, it does not seem there are significant differences between model shellfish and shellfish samples that should inhibit the detection process from being similarly successful when applied to shellfish samples.

When proposing HSI as a method for MP detection, it is necessary to discuss other documented methods of MP detection. Due to the unreactive nature of most plastics, the most common MP detection methods are largely based on physical characteristics rather than chemical properties. The most successful of these methods are optical in nature, which also have the advantage of being nondestructive. Some of these methods include Fourier Transform Infrared Spectroscopy (FTIR), Raman spectroscopy, and scanning electron microscopy (Sridhar et al.,

2022). Traditional spectroscopic methods such as FTIR and Raman Spectroscopy suffer from high monetary and labor cost (Piarulli et al., 2022), which is why HSI has been proposed and investigated as a potential replacement for these techniques.

MPs typically have reflectance spectra that appear characteristic above 1000 nm (Masoumi et al., 2012). This is outside the range of the HS camera used in this study. Therefore, this study aims to determine if there are any characteristic wavelengths or patterns in the spectral range of the camera used (400-1000nm) such that HSI could serve as a practical application to detecting MPs in shellfish meant for human consumption.

Materials and Methods

Materials

MPs were purchased from Lab 261 (Lab 261; Palo Alto, CA). Two types of red-dyed polystyrene (PS) microspheres suspended in a 5 mL solution with average diameters of 4.77 μm and 20.15 μm were purchased. Additionally, microparticles of polyvinyl chloride (PVC) with an average diameter of 5 μm were purchased. These plastics were utilized due to their presence in shellfish (Hantoro et al., 2019). The sizing of the MPs used in this study mimicked the sizing of MPs found in shellfish, which range from 5μm to 4.7mm, with the majority of MPs in mussels sizing in the range of 5 μm to 250 μm (Li et al., 2018).

For the gelatin shellfish models, Unflavored Beef Gelatin Powder (NuNaturals; Eugene, OR) was mixed with deionized water. Gelatin was chosen to model shellfish for its low viscosity, cloudy appearance, and high protein content. This gelatin powder is approximately 90% protein by weight. The solution was poured into a mold containing 16 oval cavities. The mold used was a 16 Oval Cavity Silicone Mold (X-Haibei; Chengdu City, Sichuan Province), and each cavity measured 53.3x30.5x17.8-mm per cavity. These molds resemble the common shape and size of a shellfish, as one study found mussels to be an average of 77x30x22-mm in size (Fuentes et al., 2009).

A Headwall Nano-Hyperspec (Headwall Photonics; Boston, MA) hyperspectral sensor was utilized to capture the hyperspectral images. The spectral range of this camera was 400-1000 nm, with 269 wavebands corresponding to this range. The Scanning Plant internet Of Things (SPOT) facility consists of this hyperspectral camera connected to 4 halogen lights (250 W) that move

according to the track in SPOT. The facility also has a white reflectance panel for calibration of the reflectance data. This is set up to be at the same height as the samples. The samples were placed on a piece of corrugated fiberboard on top of a black table. Figure 1 shows the SPOT facility with the HSI camera, reflectance panel, and black table. A closer look at the camera with the lights attached is displayed in Figure 2.

Figure 1. SPOT Facility Including Reflectance Panel, Camera, Lights, and Table

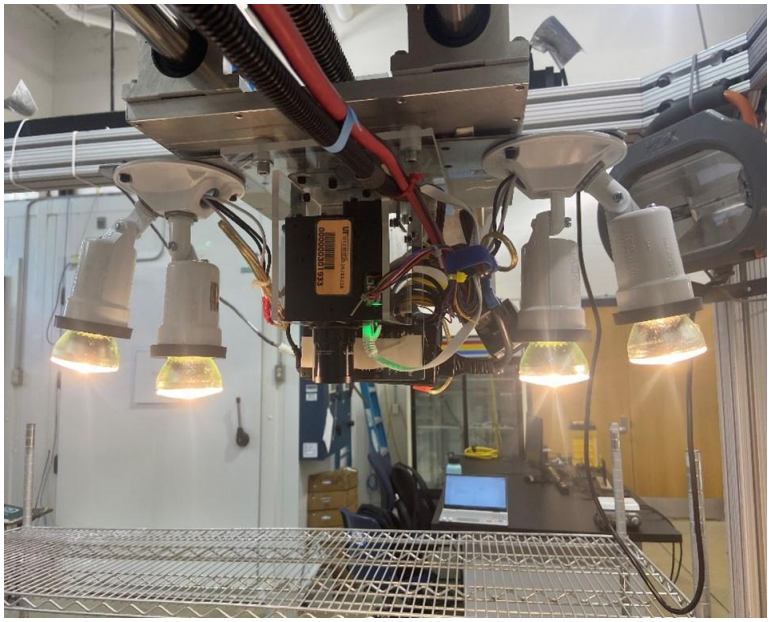

Figure 2. Camera and Lighting Setup

Methods

sample preparation. The gelatin models were prepared such that they had a protein composition of between 7.95% and 10.13% by weight to model shellfish protein content (Erkan et al., 2010). This was done by combining gelatin, which was 90% protein by weight, with water. The gelatin was added to 25% of the water which was at room temperature. This was allowed to coagulate while the other 75% of the water was boiled. Once boiled, the gelatin-water mixture was stirred, and the boiling water was added. The solution beaker was placed on a stirring plate with a small stir bar, which was set to the highest setting before a vortex formed in the center. This was done to mix the solution while minimizing trapped air. Once homogenous, the solution was poured equally into the molds. Each mold was then left at room temperature to congeal for at least 12 hours, while covered with aluminum foil.

A total of 64 gelatin models were prepared for this study. There were 16 models for each of the following treatment groups: control (no MPs were injected), models injected with PS solution (4.77μm average diameter), models injected with PS solution (20.15μm average diameter), and models injected with PVC solution (5μm average diameter). For each of the treatment groups that contained microplastic injections, 4 gelatin models were injected with 1μL of the microplastic suspension, 4 models were injected with 2 μL of the microplastic suspension, 4 models were injected with 4μL of microplastic suspension, and 4 models were injected with 8μL of the microplastic suspension. Concentrations of MPs found in shellfish indicate that 1 microplastic per gram of shellfish is commonly found (Zhang et al., 2022). However, due to limitations in pipette volumes available, the injected samples in this study contain no less than 1 μL of microplastic suspension, which equates to approximately 4774 microplastic particles per gram of sample, assuming the injected MP mass was negligible.

Injection was done using the appropriate micropipette size and pipette tip, where each set volume of microplastic suspension was added to the corresponding cavity before the cooling gelatin solution was poured. Variation in the concentration of the MPs present in the molds allowed for increased trials when testing the algorithm.

image acquisition and pre-processing. Figure 3 displays how the samples were arranged in the SPOT facility for image acquisition. Images were captured with the room lights off such that the only light source was the halogen lights, displayed in Figure 2.

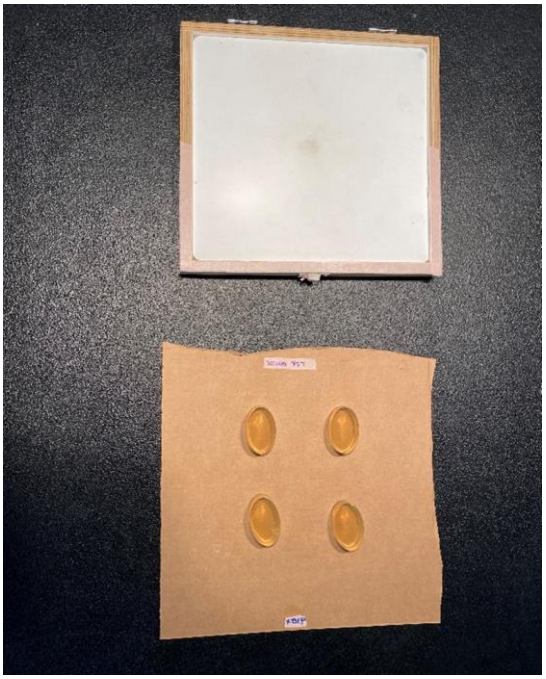

Figure 3. Gelatin Model Acquisition Arrangement

This was completed for each of the 4 microplastic concentrations of each of the 4 treatment groups (for the control, 4 models were still scanned at one time, but the microplastic concentration remained constant at 0 MPs per sample in each of the images). Therefore, the hyperspectral camera captured a total of 16 images.

The camera was controlled via a Python script in the SPOT facility and sent the data to the Hyperspec III software. Using this software, the reflectance panel was used as the white reference so that the HS hypercube information could be translated into reflectance data for comparison.

The images were exported from Hyperspec III as high dynamic range (HDR) files and then cropped using the ENVI 5.6 software. This allowed for only pixels containing shellfish models to be saved. After cropping, the images were exported as comma-separated value (CSV) files, which contained the coordinates of each pixel, and the reflectance values at each of the 269 wavebands.

algorithm development and data analysis. Using RStudio version 4.2.2, a Partial Least Squares Regression Analysis (PLSR) model was developed in the programing language R. This multivariate, linear regression modeling method was chosen due to its simplicity and ability to determine statistical properties (Cheng et al., 2016). PLSR can analyze multiple X-variables with high correlation and noise while also modeling multiple Y-variables (Wold et al., 2001). Therefore,

a PLSR model is useful in cases where there are multiple variables, but relatively few samples (Mevik & Wehrens, 2022).

Because the concentration of MPs in each sample was known, the HS data and the concentration for each image were analyzed for correlation. After images were imported into RStudio as a CSV from ENVI, they were made into data frames and assigned the correct concentration value, which acted as the "Y" variable in the PLSR. The "X" variable corresponds to the reflectance data at each of the wavelengths for each pixel. Then, 10% of the data from each sample was randomly selected to be in the combined data frame to decrease the amount of data to be analyzed, which increased the efficiency of the algorithm. This 10% was then input to the "model" function available in pls.

The PLSR package from CRAN, or the Comprehensive R Archive Network, (Mevik &Wehrens, 2022), called simply "pls", creates a PLSR model to explain variance in the 1-dimensional "Y" variable based on the data in the 2-dimensional "X" variable. The PLSR model can be validated with Root Mean Square Error of Prediction (RMSEP) using 50 leave-one-out segments to determine the number of components that will be considered to best represent the dataset. The outputs of this function, shown with the "summary" function, were then recorded. Using the information from the summary, the correlation between MP and HS data can be determined. If this percentage is above 60%, then it can be concluded that HSI can be considered to be a potential tool used to predict MP concentration.

Results and Discussion

Reflection spectra for each sample were generated using the Hyperspec III software. In Figure 4-10, the spectra are displayed. The blue, horizontal curve in each graph represents the reflectance panel for reference. Each graph also contains 4 model curves: 1 curve for each gelatin model, since 4 models were captured in each image. These model curves indicate one randomly selected pixel from each model.

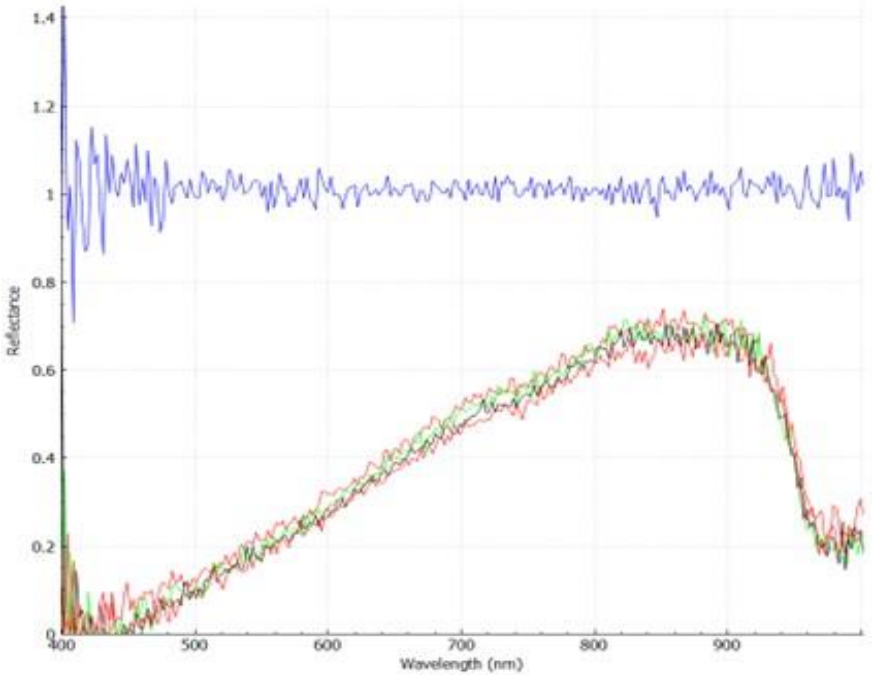

Figure 4. Reflectance Spectra of Models Without Microplastics

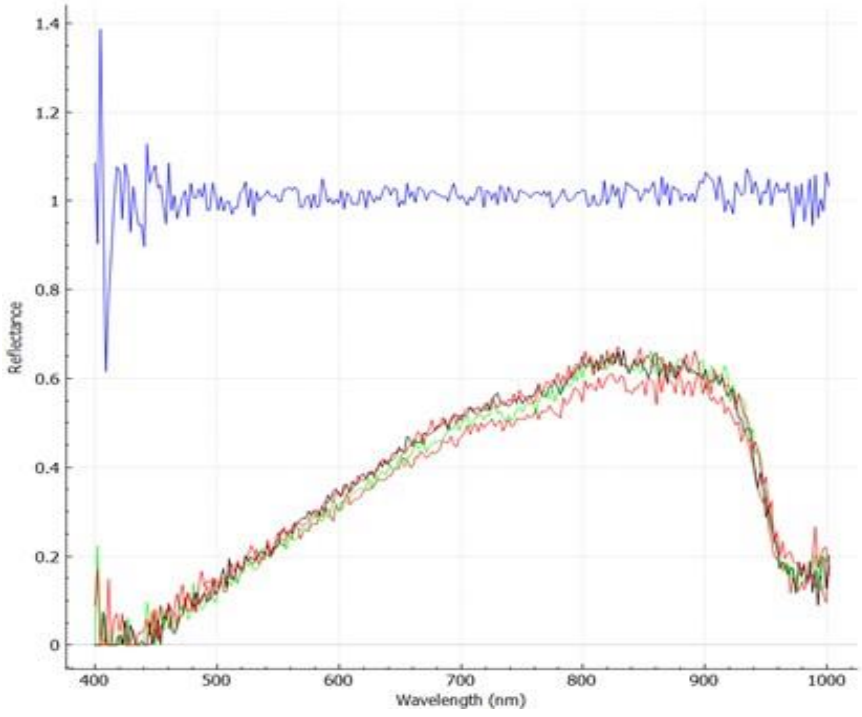

Figure 5. Reflectance Spectra of Models Injected with 1 μL PVC

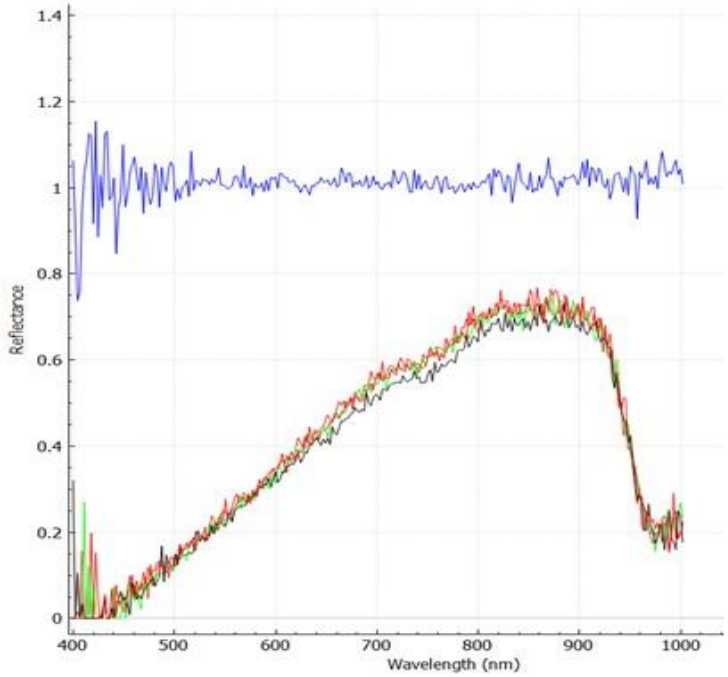

Figure 6. Reflectance Spectra of Models Injected with 8 μL PVC

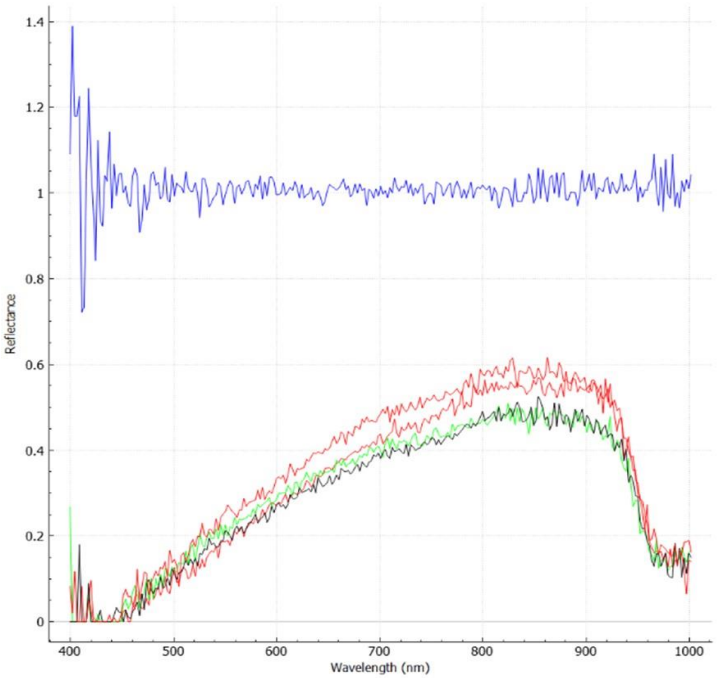

Figure 7. Reflectance Spectra of Models Injected with 1 μL PS (4.77 μm diameter)

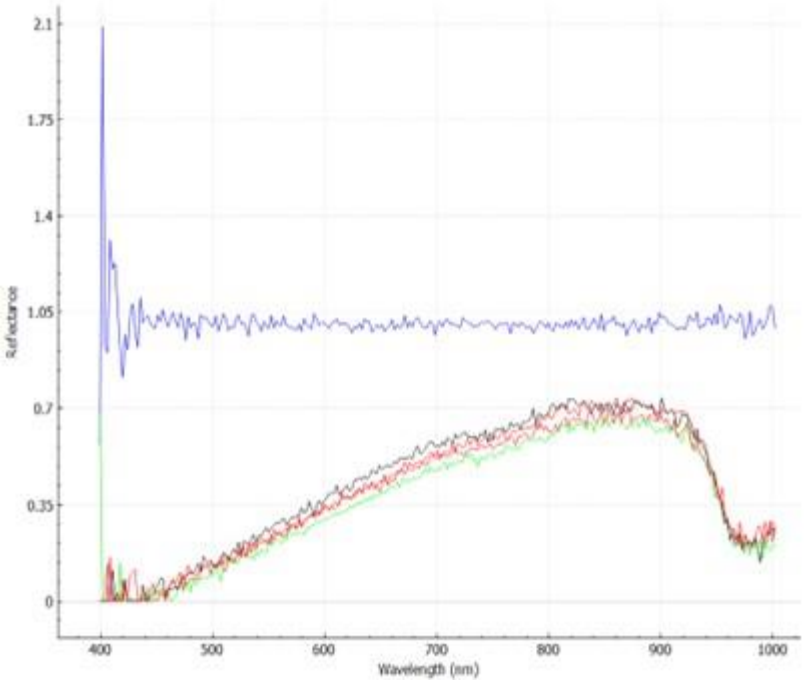

Figure 8. Reflectance Spectra of Models Injected with 8 μL PS (4.77 μm diameter)

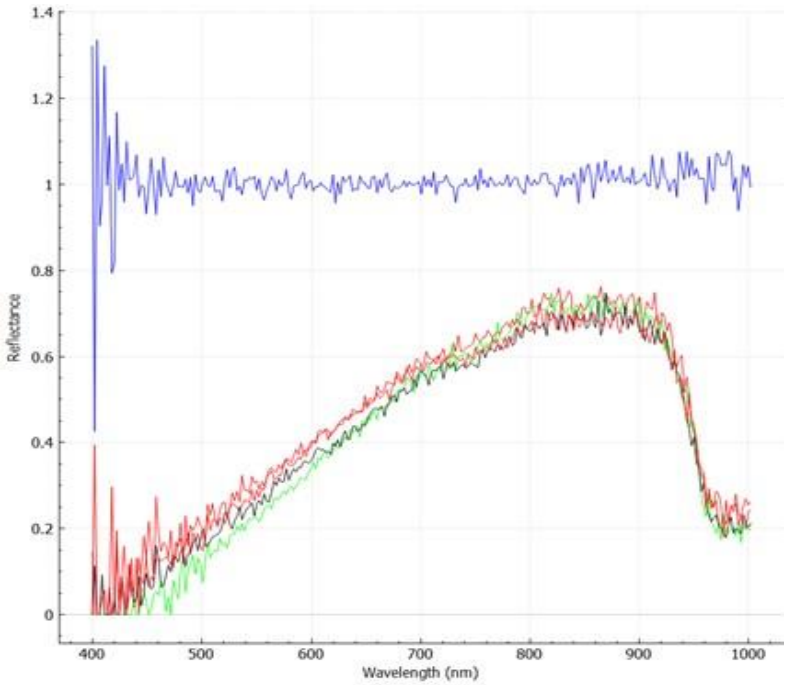

Figure 9. Reflectance Spectra of Models Injected with 1 μL PS (20.15 μm diameter)

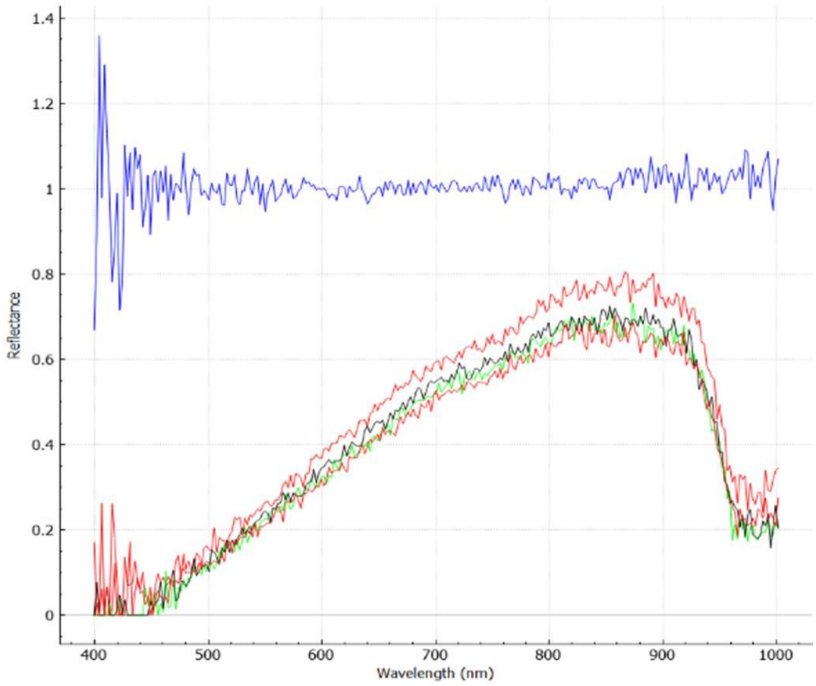

Figure 10. Reflectance Spectra of Models Injected with 8 μL PS (20.15 μm diameter)

Visual analysis of Figures 4-10 reveals that there is relatively little visual difference in the spectra of the control models, PVC models, and PS models when the reference panel is taken into account.

The algorithm was used to determine if there are differences in the reflectance from the models that the eye cannot see. After running the PLSR algorithm, it was found that the maximum percent variance in concentration explained by HS data was 6.38%. This percent variance explained occurred after 31 components, thus the algorithm could be run in the future using only 31 components instead of the original number of 50 components as there is no recorded difference after 31. The results from the PLSR analysis run with 50 components are displayed in Table 1. A PLSR was also run with 5 components and included 28224 leave-one-out segments, the summary of which can be seen in Table 2. The analysis run of 50 components did not include cross-validation, due to limitations on computing power used.

Table 1. Resulting Output from 50 Component PLSR Analysis

Number of Components Considered: 50

TRAINING: % Variance Explained

Number of components	1	2	3	4	5	6	7	8	9	10
X	90.459	96.674	97.804	99.072	99.185	99.358	99.554	99.611	99.628	99.651
Concentration	2.035	2.308	2.651	2.758	3.127	3.271	3.397	3.726	4.404	4.718
Number of components	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
X	99.651	99.700	99.724	99.744	99.757	99.770	99.776	99.789	99.799	99.810
Concentration	5.049	5.261	5.475	5.672	5.871	6.000	6.101	6.165	6.229	6.270
Number of components	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
X	99.814	99.820	99.826	99.831	99.837	99.844	99.848	99.853	99.857	99.861
Concentration	6.306	6.335	6.349	6.362	6.369	6.372	6.376	6.378	6.379	6.379
Number of components	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**
X	99.860	99.800	99.870	99.870	99.870	99.880	99.880	99.880	99.880	99.880
Concentration	6.380	6.380	6.380	6.380	6.380	6.380	6.380	6.380	6.380	6.380
Number of components	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**
X	99.890	99.890	99.890	99.890	99.890	99.890	99.890	99.900	99.900	99.900
Concentration	6.380	6.380	6.380	6.380	6.380	6.380	6.380	6.380	6.380	6.380

Table 2. Resulting Output from 5 Component PLSR Analysis with Validation

Number of Components Considered: 5

Number of components	1	2	3	4	5
TRAINING: % Variance Explained					
X	90.459	96.674	97.804	99.072	99.185
Concentration	2.035	2.308	2.651	2.758	3.127
Cross Validation: RMSEP					
CV	2.787	2.783	2.779	2.777	2.773
adjCV	2.787	2.783	2.779	2.777	2.773

Conclusions

According to the resulting output of the PLSR algorithm that showed a maximum percent variance explained for the MP concentration of 6.38% and the minute visual differences in the displayed spectra, it can be concluded that HSI in the visible to near-infrared (NIR) is not a viable tool for identifying MP concentrations. Some of the explained 6.38% of the variation of

the data may also be explained by the red dye in the polystyrene samples, which may not be present in abundance in microplastic sampling in the field. This means that over 93% of the variation in concentration cannot be explained using the methodology outlined above. This is likely due to plastic polymer spectra existing outside of the range of the camera. Therefore, future research in nondestructive spectroscopic methods of detecting MPs in food or other biological materials should explore cameras with higher ranges to accommodate the polymers' high spectra in the infrared range of the electromagnetic spectrum. Additionally, it could be beneficial to do preliminary scans of pure plastic, rather than models injected with plastic. This data could be used to inform researchers of the ability of their equipment to scan plastics and be used to train a PLSR or similar model.

This study represents a preliminary step in identifying nondestructive spectroscopic methods to detect MPs in shellfish meant for human consumption. Future investigation into HSI as a method for microplastic detection in shellfish is warranted. The successful implementation of such a method would allow for the identification of potentially hazardous food that contains MPs.

Acknowledgements

The authors would like to thank the University of Florida University Scholars Program (USP) for providing the funding for this project in the 2022-2023 term. The authors would also like to thank the mentor for all the support and guidance granted. A special thanks to Stephen Lantin for helping every step of the way and to Nathan O'Neil for lending his expertise in the development of physical seafood models. The authors would also like to thank Dr. Aditya Singh for providing access to the SPOT facility and other laboratories used to complete this research.

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