PROXIMATE COMPOSITION AND FATTY ACID PROFILE OF SOUTH AFRICAN SARDINES (*SARDINOPS SAGAX OCELLATUS*) USING CONVENTIONAL TECHNIQUES AND NEAR INFRARED (NIR) SPECTROSCOPY

BY

ZIYANDA PHIKE

Thesis presented in partial fulfillment of the requirements for the degree

OF

Master of Food Science and Technology

In the Faculty of Applied Sciences

At Cape Peninsula University of Technology

Supervisor: Dr S. Henning, CPUT

Bellville

November 2020

DECLARATION

I, the undersigned, declare that the work contained in this thesis is my own, original work, that it has not previously been submitted at any university for obtaining any qualification.

Signed Date: 17 Feb 2021

ABSTRACT

Fish and fishery products form an essential part of a healthy human diet due to the high-quality proteins and omega-3 fatty acids associated with it. The South African (SA) sardine (*Sardinops sagax ocellatus*) is commercially important in South Africa's purse seine fishery and the fish canning industry. Reasons being sardines are an important source of high-quality proteins and fatty acids and therefore used as a food source for humans and pets. Moreover, sardine fishery offers employment to a large SA workforce. The growing consumption of sardines has led to the fish canning industry investigating effective instrumental techniques of evaluating the quality of the respective products.

Multivariate data analysis, in particular principal component analysis (PCA), was applied on acquired near infrared (NIR) spectra to investigate the correlation between SA sardines based on morphophysiological properties. Sample sets of whole (n=116), and homogenised (n=97) sardine samples were investigated based on sex, fat tage and gonad stage, using PCA. For the whole fish sample set, groupings of sex, fat and gonad stages were distinguishable. Homogenised fish samples could not be classified based on sex and gonad stages. Only the fat stages could be differentiated when using the homogenised fish samples.

Homogenized sardine samples were scanned by NIR. Sub samples were used to determine percentage moisture, ash, protein and fat using conventional methods. The data sets from the NIR scans and from the chemical analysis were combined to develop calibration models to allow prediction of proximate composition. These results were used as reference values to build partial least square (PLS) regression models using the NIR data-spectra. Prediction statistics for the respective validation sets were: R^2_v = 0.22, SEP = 3.14%, RPD 1.15 for moisture, R^2_v = 0.24, SEP = 0.056%, RPD 1.07 for ash, R^2_v = 0.61, SEP = 2.46%, RPD 1.47 for fat and R^2_v = 0.22, SEP = 3.41%, RPD 1.07 for protein. The results indicated that no reasonable predictions could be made at the fast NIR scan rate.

Additionally, the fatty acid composition of sardines was obtained using a conventional gas chromatography (GC) method. These results were used to build PLS regression models in combination with NIR spectra acquired from the respective sample sets. The developed PLS models resulted in poor predictions for saturated fatty acids (SFA): R^2_v = 0.48, SEP = 0.45% and RPD = 0.65 and polyunsaturated fatty acids (PUFA): R^2_v = 0.48, SEP = 0.44% and RPD = 1.47. Monounsaturated fatty acids (MUFA) had very poor prediction results with very little correlation found between the predicted and reference values (R^2_v = 0.38, SEC = 2.33). Likewise, total fatty acids resulted in poor predictions (R^2_v = 0.37, SEC = 2.03). NIR spectroscopy has potential to be

used as an analytical technique to predict proximate and fatty acid composition of fish, however further improvement is still necessary before it can be considered for industrial use.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to the following people and institutions that formed an integral part of the completion of this research study:

Dr Suné St Clair Henning, my supervisor at the Cape Peninsula University of Technology, for her patience, support and assistance during the course of my studies;

Dr Anina Guelpa for reviewing my work and providing feedback;

My co-supervisor, Professor Manley for reviewing my work and providing feedback;

Staff members and post graduate students at the Department of Food Science and Technology, CPUT;

Mr Ndumiso Mshicileli and Malibongwe Menziwa at Agrifoods Technology Station for helping with fatty acids analysis;

Staff members at the Department of Animal Science, Stellenbosch University: Lisa Uys, Beverley Ellis for the use of their facility for fat and protein analysis;

Department of Agriculture, Forestry and Fisheries: Dr Carl Van der Lingen and Dumisani Ntiyantiya for the provision of fish samples and helping with morphophysiological analysis;

Food & Beverage SETA and Wholesale & Retail SETA for financial assistance;

National Research Foundation and Agrifoods Technology Station for their financial support through internships

My siblings who always have my best interest at heart, to my mother for the years spent looking after my daughter when I couldn't

My sister, my friend Portia Griffiths for her constant moral support and for the happy distractions to rest my mind outside of my research.

DEDICATION

Dedicated to the memory of my late father Lungiselo Phike (†05.05.2003)

Dedicated to the memory of my late brother Thembela Phike (†19.04.2009)

Dedicated to the memory of my late grandmother Nonkululeko Ndibeni (†03.10.2010)

GLOSSARY

Terms/Acronyms/Abbreviated	Definition/Explanation
NIR	Near infrared
PCA	Principal component analysis
PLS	Partial least squares
AOAC	Association of official analytical chemists
PUFA	Polyunsaturated fatty acids
MUFA	Monounsaturated fatty acids
SFA	Saturated fatty acids
PA	Palmitic acid
LA	Lauric acid
MA	Myristic acid
EPA	Eicosapantaenoic acid
DHA	Docosahexaenoic acid
ALA	Alpha-linolenic acid
DPA	Docosapentaenoic acid
PPARs	Peroxisome proliferator-activated receptors
SREBP	Sterol regulatory element binding protein
KCL	Potassium chloride
LVF	Linear variable filter
NMR	Nuclear magnetic filter
MDA	Multivariate data analysis
PC	Principal component
RMSEP	Root mean square error of prediction
RMSECV	Root mean square error of cross-validation
RPD	Ratio performance deviation
SD	Standard deviation
SECV	Standard error of cross-validation
SEC	Standard error of calibration
MSC	Multiplicative scatter correction
SG	Savitzky Golay
R^2_C	Coefficient of determination for calibration
R^2_V	Coefficient of determination for validation

DAFF Department of Agriculture, Forestry and Fisheries now renamed to	Department of Agriculture Land Reform and Rural Development (DALRRD; 2020)
CPUT	Cape Peninsula University of Technology
CL	Caudal length
Cm	Centimeter
PFTE	Polytetrafluoroethylene
EDTA	Ethylene diamine tetraacetic acid
HPLC	High performance liquid chromatography
GC	Gas chromatography
TLC	Thin layer chromatography
UV	Ultraviolet
FAME	Fatty acid methyl esters
SIMCA	Soft independent modelling of class analogy
PCA-R	Principal component analysis regression

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

1 CHAPTER ONE
MOTIVATION AND DESIGN OF THE STUDY

1.1 Introduction

Canned fish and processed fish products are of high economic importance in many countries (Aubourg & Medina, 1997). In general, fish and fishery products have long been recognised to be essential parts of a healthy human diet and are considered necessary for human health due to the high-quality protein content, vitamin B and D content, minerals, and omega-3 fatty acids thereof (Aubourg, 2001; Cheng *et al*., 2013; Shingadia, 2013; Blakistone *et al*., 2016; Jim *et al*., 2017). Canned fish and fishery products are an important food source and have major advantages over fresh and frozen fish (Moody, 2003; Selmi *et al*., 2008). Canned fish products have advantages such as convenience, safety and the relatively long shelf life at ambient temperatures (Moody, 2003; Selmi *et al*., 2008).

Knowledge of fish proximate composition is very crucial in implementing the correct processing parameters during production (Yeannes & Almendos, 2003). The determination of proximate composition and other nutrients of fish has raised great concern about the quality of canned foods in different parts of the world (Odiko & Obirenfoju, 2017). The quantification of proximate composition and other nutrients is necessary to ensure compliance with dietary requirements and commercial specifications (Jim *et al*., 2017; Odiko & Obirenfoju, 2017). Information pertaining to the changes that occur in canned fish flesh from the early processing stages is limited (Selmi *et al*., 2008).

South African (SA) sardines *(Sardinops sagax ocellatus)*, also popularly known as pilchards (Grant *et al*., 1998), are small pelagic fish species that belong to the Clupeidae family (Van der Lingen *et al.,* 2006). The SA sardine is among the thirteen species that occur in the SA marine ecosystem (Whitehead, 1985). Sardines significantly contribute towards the purse seine catches in the northern and southern Benguela Current upwelling ecosystem (Van der Lingen & Durholtz, 2003). In essence, sardines are one of the most ecological and economic important fish in the Benguela ecosystem, sustaining a significant number of fisheries that employ a substantial number of people (Mhlongo *et al.,* 2015).

The commercial harvest of SA sardine has been continuing since the 1940s in St Helena Bay, with two periods of peak catches (Beckley & Van der Lingen, 1999; Van der Lingen *et al*., 2014). The first catch peak at around (400,000 t) occurred in 1962 (Crawford *et al*., 1987; Coetzee *et al*., 2008), followed by a second peak of over 250 000 t in 2002 to 2004 (De Moor & Butterworth,

2009). The reduction in SA sardine catches has led to the importation of frozen sardine by local fish processing companies and processing of those fish to meet the domestic demand for canned sardine (Macey *et al*., 2016).

SA sardines do not seem to have a fixed spawning area and have been reported to spawn along almost their entire distribution range (Anders, 1975; Armstrong *et al*., 1991; Barange *et al*., 1999; Van der Lingen & Huggett, 2003). SA sardines start spawning from the age of two years (Armstrong & Thomas, 1989; Van der Lingen 1994; Zlatanos & Laskaridis, 2007). The accumulated energy from food intake in juvenile sardines is used for growth, whereas in adult sardines lipids are used primarily for gonad development (Davies 1956; Patterson 1992; Adams *et al.,* 1995; Van der Lingen, 1999). The high quantity and quality of food in the adult sardine life cycle is therefore vital to the health condition of the fish, especially before the spawning season (De Goede, 2004). Sardines with high lipid content values are considered to be in better condition for spawning than sardines with low lipid content values (De Goede, 2004).

Fish condition refers to the robustness and well-being of an individual fish and is believed to be a good indicator of general fitness of the population under consideration (De Goede, 2004; Sreeleshmi *et al*., 2017). Fish condition relates to a wide range of attributes including aspects of ecological performance such as the ability of fish to reproduce successfully (Yeen *et al*., 2005). Biologists have developed a wide range of morphological, biological and physiological techniques to assess fish condition (Mustać & Sinovčić, 2009). Morpho-physiological indicators are considered to be simple measures that determine the length or weight of fish and are quickly gathered by non-destructive methods (Murphy *et al*., 1990; Lloret *et al*., 2014).

Fish meat encompasses many constituents such as moisture, protein, lipids, vitamins and minerals, which contribute to the proximate composition thereof. Information on the chemical composition of fish is important for the application of different processing technologies, post-mortem characteristics of species, providing an idea of the sexual stage of the respective fish and as a quality characteristic of raw material (Huss, 1988, Yeannes & Almandos, 2003). Fish proximate composition differs among individuals and species according to the season, sex, size, life cycle stage and anatomical positions of the body (Love, 1980; Huss, 1988; Huss, 1995; Olsson *et al*., 2002 Boran & Karaçam, 2011). The time of the year is the most important factor that influences the composition of the diets that are accessible in different seasons. Fish diet is the main factor affecting proximate composition and varies with season, which leads to a seasonal variation in fat content and fatty acid concentration (Szlinder-Richert *et al*., 2010; Mohamed *et al*., 2016).

Several conventional methods are available for the determination of the proximate composition and fatty acid profile of samples (Uddin & Okazaki, 2004). Such frequently used methods are the Kjeldahl method for protein determinations, oven-drying to determine the moisture content, the Soxhlet method for fat content determinations, incineration to determine the ash content and also gas chromatography when determining the fatty acid content (Saeed *et al*., 2012). Likewise, when considering fish proximate composition as well as fatty acid profile, the above-mentioned analytical methods have been used (Bernardi *et al*., 2013). However, it should be noted that these analytical methods cannot be used for a fast-paced production and processing environment because they are tedious, expensive, time-consuming, are associated with inconsistency and variability, destructive to the sample and sometimes environmentally unfriendly due to the chemical pollution from the conventional methods (Zhang *et al*., 2000; Cozzolino *et al*., 2005; Huang *et al*., 2014; Kamruzzaman *et al*., 2015).

Alternatively, near-infrared (NIR) spectroscopy has shown to be a reliable method to determine fish chemical composition (Nortvedt *et al*., 1998; Boknaes *et al*., 2002; Kimiya *et al*., 2013). This method offers accurate and objective results and requires little or no training and can be implemented using a small handheld instrument such as the MicroNIR (O'Brien *et al*., 2013) as well as an online device for non-contact measurements of fish during production (Kimiya *et al*., 2013). The main advantages of NIR spectroscopy over the conventional methods are that it is rapid, accurate and non-destructive (Nortvedt *et al*., 1998; Osborne, 2000; Baeten & Dardenne, 2002). The technique is based on the absorption of electromagnetic radiation at wavelengths ranging between 780 and 2500 nm (Osborne, 1993). The absorption bands observed within this range result from overtones and combination bonds of oxygen-hydrogen (O-H), nitrogen-hydrogen (N-H), carbon-hydrogen (C-H) and sulphur-hydrogen (S-H) (Pasquini, 2003; ELMasry & Wold, 2008). These vibrations allow qualitative and quantitative analysis of chemical and physical characteristics of food constituents (ELMasry & Wold, 2008).

The visual examination of NIR spectra alone cannot distinguish between samples or be used for quantitative predictions, and therefore requires the application of multivariate data analysis techniques, otherwise called chemometrics (Cozzolino *et al*., 2005). Associated with chemometrics, NIR spectroscopy becomes a powerful tool in quantitative and qualitative analysis (Roggo *et al*., 2007). Chemometrics is the application of mathematical, statistical and computer science methods to improve the extraction of valuable data from chemical analysis. Principal component analysis (PCA) is an unsupervised, frequently used clustering technique that recognises and explores complex multivariate data sets (Wold *et al*., 1987; Geladi *et al*., 2004 &

Pere-Trepad *et al*., 2006). It reduces large amounts of data into principal components also known as latent variables (Massart, 1998).

Partial least squares (PLS) regression is a technique that uses the response information of two or more sets of variables in building a model (Martens & Naes, 1991; Dahlman, 2012) and is the most popular linear calibration method used for quantitative data analysis (He & Sun, 2015). PLS regression is a supervised method that reduces the complexity of an extensive data set of variables by extracting a smaller amount of new latent variables to predict a set of dependent variables (responses) from a set of independent variables (predictors) (Geladi & Kowalski, 1986; Lavine & Workman, 2005).

The purpose of this study was to use PCA applied on NIR spectra to explore patterns of similarities in the morphophysiological indicators of South African sardines *(Sardinops sagax ocellatus*) and to develop calibration models to predict proximate composition and fatty acid profile of using reference methods in combination with NIR spectra and PLS regression. The usefulness of the more rapid NIR spectroscopy technique was compared to that of conventional techniques.

1.2 Statement of the research problem

The quality of the data collected by fisheries has great impact on the quality of stock assessment and is of importance to marine resource management. It is crucial that the SA pelagic fishery gathers information on the morphophysiological characteristics, proximate composition and fatty acid profile of sardines. There are several conventional methods that could evaluate morphophyisiological indicators, proximate composition and fatty acid profile of fish. However, these measurements are considered to be time-consuming and quite costly. Therefore, an alternative method, i.e. NIR spectroscopy, was investigated as a possible alternative to conventional techniques as this method is claimed to be fast, non-destructive and relatively low cost once calibrated. The usefulness of the developed NIR spectroscopy models for proximate composition and fatty acid profile of SA sardines was compared to that of conventional methods.

1.3 Broad objective

The purpose of the study was to obtain NIR calibration models to predict proximate composition and fatty acid profile of SA sardines based on NIR spectra. The developed models were used for the quantification of the respective components. Conventional NIR instruments are bench-top laboratory-based instruments. Usually, the majority of analyses are performed by taking samples

from the production site and transporting it to the quality control laboratory that is separately situated. Consequently, there is a delay between sampling and getting the results, which limits the regularity of analysis. But recently, with the development of portable NIR spectrophotometers capable of performing rapid, at line or in line analysis, these are seen as practical quality control tools.

1.4 Specific objectives

- The first objective was to explore the sex and morphophysiological indicators (fat stage, gonad stage) of whole SA sardine samples scanned on the skin as well as homogenised sardines using the acquired NIR spectral data and principal component analysis (PCA
- The third objective was to develop models to predict proximate composition (crude fat, protein, and moisture) of the SA sardine using the acquired NIR spectral data and PLS regression models.
- The last objective was to develop models to predict the fatty acid composition of the South African sardine using the acquired NIR spectral data and PLS regression models.

1.5 Hypothesis

Previous research (Gjerde & Martens, 1987; Issakson *et al*., 1995; Khodabux *et al*., 2007; Wold & Issakson, 1997) has indicated that proximate composition, as well as fatty acid profile of fish, can be successfully predicted using conventional methods in combination with NIR spectroscopy. No similar studies have been performed on the SA sardine. It was therefore hypothesised that NIR spectroscopy and PLS regression in combination with reference methods will be successful in predicting proximate composition as well as the fatty acid profile of SA sardine, thereby replacing conventional, time-consuming methods.

1.6 Delineations of the research

- SA sardine (*Sardinops sagax ocellatus*) was analysed during this study.
- Only whole (intact) and homogenised fish samples as opposed to filleted fish were considered in this study.

- Sex and morphophysiological indicators (fat stage and gonad stage), proximate (moisture, ash, protein and fat) composition and fatty acid profile were analysed.
- Only a hand-held NIR instrument (MicroNIR) was used in this study.

1.7 Significance of the study

NIR spectroscopy offers several advantages over various conventional analytical methods. It allows fast, non-destructive, non-invasive and real-time quality control measures, saving money on the long-term since chemical analysis is expensive and time-consuming (Karoui & de Baerdermaeker, 2007; Cheng *et al*., 2013). The use of NIR instrument minimizes chemical pollution associated with conventional methods (Zhang *et al*., 2000). Keeping all mentioned reasons in mind, developing NIR calibration models with sufficiently accurate prediction power for morphophysiological indicators, proximate and fatty acid analysis using a portable hand-held instrument will significantly benefit the SA fisheries, research and management.

1.8 Thesis Overview

The thesis was compiled using an article format, consisting of six chapters where each research chapter is an individual entity. A list of references is cited at the end of each chapter. Chapter one gives the motivation and design of the study, stating the research problem, objectives, hypothesis, delineations, contributions as well as expected outcomes, results and contributions to the study. Chapter two is the literature review which expands on the background of the research topic. A background on fish consumption and its associated benefits was highlighted; the importance of the sardine species in the South African purse-seine fishery was reviewed followed by the proximate composition and nutritional importance of fish. Conventional methods used for the determination of fish constituents were discussed. Finally, the application of NIR spectroscopy coupled with reference methods as an alternative method for the prediction of fish proximate composition was reviewed. Chapter three is the first research chapter detailing the application of NIR spectroscopy and PCA to the exploration morphophysiological indicators of whole as well as homogenised SA sardines. In this chapter, PCA was applied to the NIR spectral data to group sardines according to the mentioned indicators. Chapter four is the second research chapter focusing on the prediction of proximate composition of SA sardines using reference methods with NIR spectral data appropriate chemometrics to develop prediction models. Proximate composition

(moisture, protein, fat and ash) were analysed using AOAC methods to obtain results for PLS regression modelling. Chapter five is the final research chapter and focused on generating PLSR calibration model for the use of routine fatty acid analysis in SA sardine. The fatty acid profile of SA sardines was determined using AOAC and PLS regression methods. Chapter six gives the general discussion and conclusion of the study.

References

Adams, C.E., Huntingford, F.A. & Jobling, M. (1995). A non-destructive morphometric technique for estimation of body and mesenteric lipid in Arctic charr: a case study of its application. *Journal of Fish Biology*, **47**, 82-90.

Armstrong, M.J. & Thomas, R.M. (1989). Clupeoids. In: *Oceans of life* (edited by A.I.L. Payne & R.J.M. Crawford). Pp. 105-121. Publishers: Vlaeberg. Cape Town.

Armstrong, M.J., Chapman, P., Dudley, S.F.J., Hampton, I. & Malan, P.E. (1991). Occurrence and population structure of pilchard *Sardinops ocellatus*, round herring *Etrumeus whiteheadi* and anchovy *Engraulis capensis* off the east coast of southern Africa. *South African Journal of Marine* Science, **11**, 227-249.

Aubourg, S.P. & Medina, I. (1997). Quality differences assessment in canned sardine (*Sardina pilchardus*) by fluorescence detection. *Journal of Agricultural and Food Chemistry*, **45**, 3617-3621.

Aubourg, S.P. (2001). Loss of quality during the manufacture of canned fish products. *Food Science and Technology International*, **7**(3), 199-215.

Baeten, V. & Dardenne, P. (2002). Spectroscopy: Developments in instruments and analysis. *Grasas y Aceites*, **53**, 45-63.

Barange, M. and Coetzee, J. (1999). Results of the 1999 pelagic recruitment survey. *Marine and Coastal Management*, Department of Environmental Affairs and Tourism, WG/JUN99/PEL/19 (unpublished).

Bernardi, D.C., Marsico, E.T. & Queiroz de Freitas, M. (2013). Quality index method (QIM) to assess the freshness and shelf life of fish. *Brazilian Archives of Biology and Technology*: *An International Journal*, **5**(4). 587-598.

Blakistone, I., Kleiner, R. & McGuire, J. (2016). Fish: Fish in the Human Diet. *Reference Module in Food Science. Encyclopedia of Food and Health*, 706-709.

Boknaes, N., Jensen, K. N., Anderson, C. M. & Martens, H. (2002). Freshness assessment of thawed and chilled cod fillets packed in modified atmosphere using near-infrared spectroscopy. *Food Chemistry*, **35**, 628-634.

Boran, G. & Karaçam, H. (2011). Seasonal changes in proximate composition of some fish species from the Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, **11**, 1-5.

Cheng, J., Sun, D., Zeng, X. & Liu, D. (2013). Recent advances in methods and techniques for freshness quality determination and evaluation of fish and fish fillets: A Review. *Food Science and Nutrition*, **55**(7), 1012-1226.

Coetzee, J.C., Van der Lingen, C.D., Hutchings, L., Fairweather, T.P., 2008. Has the fishery contributed to a major shift in the distribution of South African sardine? *ICES Journal of Marine Science*, **31**(9), 333–348.

Cozzolino, D., Chree, A., Scaife, J.R. & Murray, I. (2005). Usefulness of near-infrared reflectance (NIR) spectroscopy and chemometrics to discriminate fishmeal batches made with different fish species. *Journal of Agricultural and Food Chemistry*, **53**, 4459-4463.

Crawford, R.J.M., Shannon, L.V. & Pollock, D.E. (1987). The Benguela ecosystem Part IV. The major fish and invertebrate resources. *Oceanography Marine Biology Ann*, **25**, 353–505.

Dahlman, N. (2012). Near infrared spectroscopy- An introductory study on measurement techniques and tools for analysing content in pulp and paper media. Master of Science in Chemical Engineering. Karlstads University. Sweden.

Davies, D.H. (1956). The South African pilchard (*Sardinops ocellata*) sexual maturity and reproduction 1950-1954. Investigational Report Division of Sea Fisheries, South Africa, **22**, 1-55.

De Goede, J. (2004). Spatial and temporal variability in lipid content and gonadosomatic index of sardine (*Sardinops sagax*) in Southern Benguela ecosystem. Master of Technology Thesis. Cape Technikon. South Africa.

De Moor C.L. & Butterworth D.S. (2009). The 2004 re-assessment of the South African sardine and anchovy populations to take account of revisions to earlier data and recent record abundances. *African Journal of Marine Science*, **31**, 333–348.

Elmasry, G. & Wold, P.J. (2008). High speed assessment of fat and water content distribution in fish fillets using online imaging spectroscopy. *Journal of Agricultural and Food Chemistry*, **56**, 7672-7677.

Geladi, P. & Kowalski, B. R. (1986). An example of a 2-block predictive partial least squares regression with simulated data. *Analytica Acta*, **185**, 19-32.

Geladi, P., Burger, J. & Lestander, T. (2004). Hyperspectral imaging: calibration problems and solutions. *Chemometrics and Intelligent Laboratory Systems*, **72**, 209-217.

Gjerde, B. & Martens, H. (1987). Predicting carcass composition of rainbow trout by near-infrared reflectance spectroscopy. *Journal of Animal Breed and Genetics*, **104**, 137-148.

Grant, W.S., Clark, A.M. & Bowen, B.W. (1998). Why restriction fragment length polymorphism analysis of mitochondrial DNA failed to resolve sardine (*Sardinops*) biogrography: insights from mitochondrial DNA cytochrome *b* sequences. *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 2539-2547.

He, H. & Sun, D. (2015). Toward enhancement in predictions of Pseudomonas counts distribution in salmon fillets using NIR hyperspectral imaging. *LWT-Food Science and Technology*, **62**, 11-18.

Huang, H., Liu, L. & Ngadi, M.O. (2014). Recent Developments in Hyperspectral Imaging for Assessment of Food Quality and Safety. *Sensors*, **14**, 7248-7276.

Huss, H.H. (1988). Chemical composition. In: *Fresh fish: quality and quality changes*. Pp. 15-25. Rome: Food and Agriculture Organization of the United Danish International Development Agency.

Huss, H.H. (1995). Quality and quality changes in fresh fish. In: *Fresh fish: quality and quality changes*. Food and Agriculture Organization Technical Paper 348, FAO, Rome.

Isaksson, T., Tøgersen, G., Iversen, A, & Hildrum. K.I. (1995). Nondestructive determination of fat, moisture and protein in salmon fillets by use of near-infrared diffuse spectroscopy. *Journal of the Science of Food and Agriculture*, **69**, 95-100.

Jim, F., Garamumhango, P. & Musara, C. (2017). Comparative analysis of nutritional value of *Oreochromis niloticus* (Linnaeus), Nile Tilapia, meat from three different ecosystems. *Journal of Food Quality*, **2017**, 1-8.

Kamruzzaman, M., Makino, Y. & Oshita, S. (2015). Non-invasive analytical technology for the detection of contamination, adulteration and authenticity of meat, poultry and fish: A review. *Analytica Chimica Acta*, **853**, 19-29.

Karoui, R. & de Baerdermaeker, J. (2007). A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products. *Food Chemistry*, **102**, 621-640.

Khodabux, K., L'Omelette, M.S.S., Jhaumeer-Laullo, S., Ramasami, P. & Rondeau, P. (2007). Chemical and near-infrared determination of moisture, fat and protein in tuna fishes. *Food Chemistry*, **102**, 669-670.

Kimiya, T., Sivertsen, A. H. & Heia, K. (2013). Vis/NIR spectroscopy for non-destructive freshness assessment of Atlantic salmon (*Salmo salar*) fillets. *Journal of Food Engineering*, **116**, 758-764.

Lavine, B.K. & Workman, J.J. (2005). Chemometrics: Past, present and future. In: *Chemometrics and chemoinformatics* (edited by B.K. Lavine). Pp. 1-13. Washington, DC: American Chemical Society.

Lloret, J., Shulman, G. & Love, R.M. (2014). Description of condition indicators. In: Condition and health indicators of exploited marine fishes. John Wiley & Sons Ltd. Chichester. USA

Love, R.M. (1980). The chemical biology of fishes. Pp. 387. London: Academic Press.

Macey, B.M., Christison, K.W., De Goede, J., Hutchings, L. & Van der Lingen, C.D. (2016). Testing the occurrence of pilchard herpesvirus (PHV) in South African sardine *Sardinops sagax*. *African Journal of Marine Science*, **38**(2), 1-5.

Manley, M., McGoverin, C.M., Snyders, F., Muller, N., Botes, W.C. & Fox, G.P. (2013). Prediction of triticale grain quality properties based on both chemical and indirectly measured references methods using near-infrared spectroscopy. *Cereal Chemistry*. **90** (6), 540-545.

Martens, H. & Naes, T. (1991). *Multivariate calibration*. New York, USA: John Wiley & Sons Ltd.

Mhlongo, N., Yemane, D., Hendricks, M. & Van der Lingen, C.D. (2015). Have the spawning habitat preferences of anchovy (*Engraulis encrasicolus*) and sardine (*Sardinops sagax*) in the Southern Benguela changed in recent years? *Fisheries Oceanography*, **24**(1), 1-14.

Mohamed, F.A., Khogali, F.A., Mohamed, A.H., Deng, O.O. & Mohammed, A.A. (2016). Body weight characteristics and chemical composition of Nile tilapia *Oreochromis niloticus* collected from three different Sudanese dams. *International Journal of Fisheries and Aquatic Studies*, **4**(5), 507-510.

Moody, M.W. (2003). Fish Processing. In: Encyclopedia of Food Sciences and Nutrition (edited by L. Trugo and P.M. Finglas). (2nd Edition) Pp. 2453- 2457, Elsevier Science Ltd. USA.

Murphy, B.R., Brown, M.L. & Springer, T.A. (1990). Evaluation of the relative weight (W_r) index with new applications to walleye. *North American Journal of Fisheries Management*, **10**(1), 85-97

Mustać, B. & Sinovčić, G. (2009). Comparison of mesenteric and tissue fat in relation to sexual cycle of the sardine, *Sardina pilcardus* (Walb., 1972), in the eastern Middle Adriatic fishery grounds (Croatia). *Journal of Applied Ichthyology*, **25**, 595-599.

Nortvedt, R., Torrissen, O.J. & Tuene, S. (1998). Application of near-infrared transmittance spectroscopy in the determination of fat, protein and dry matter in Atlantic halibut fillet. *Chemometrics and Intelligent Laboratory Systems*, **42**, 199-207.

Odiko, A.E. & Obirenfoju, J. (2017). Proximate composition and mineral contents of different brands of canned fishes marketed in Edo State Nigeria. International *Journal of Fisheries and Aquaculture Research*, **3**(2), 30-38.

Olsson, G.B., Olsen, R.L., Carlehog, M., Ofstad, R. (2002). Seasonal variation in chemical variation and sensory characteristics of farmed and wild Atlantic halibut (*Hippoglossus hippoglossus*), *Aquaculture*, **217**, 191-205.

Osborne, G.B. (1993). Near-infrared spectroscopy in Food analysis. In: *Encyclopedia of analytical chemistry* (edited by R. A. Meyers). John Wiley & Sons Ltd. Chichester.

Osborne, B.J. (2000). Near infrared spectroscopy in food analysis. In: *Encyclopedia of analytical chemistry* (edited by R.A. Meyers). John Wiley & Sons Ltd. Chichester.

Pasquini, C. (2003). Near infrared spectroscopy: Fundamentals; practical aspects and analytical applications. *Journal of Chemistry*, **14**(2). 198-219.

Patterson, K.R. (1992). An improved method of studying the condition of fish, with an example using Pacific sardine *Sardinops sagax* (Jenyns). *Journal of Fish Biology,* **40**, 821-831.

Pere-Trepad, E., Olivella, L., Ginebreda, A., Caixach, J. & Jauler, R. (2006). Chemometrics modelling of organic contaminants in fish and sediment river samples. *Science of the Total Environment*, **371**, 223-237.

Roggo, Y., Chalus, P., Maurer, L., Lema-Martinez, C., Edmand, A. & Jent, N. (2007). A review of near-infrared spectropy and chemometrics in pharmaceutical technologies. *Journal of Pharmaceuticals and Biomedical Analysis,* **44**, 683-700.

Saeed, M., Ali Reza, A., Hamid, F., Maryan, S. & Nuria, P. (2012). Determination of protein and moisture in fishmeal by near-Infrared reflectance spectroscopy and multivariate regression based on partial least squares. *Iranian Journal of Chemical Engineering,* **31**(3), 51-59.

Selmi, S., Monser, L. & Sadok, S. (2008). The influence of local canning process and storage on pelagic fish from Tunisia: fatty acid profiles and quality indicators. *Journal of Food Processing Preservation*, **32**, 443-457.

Sreelekshmi, S. Benno Pereira, F.G. & Binil, V.S. (2017). Morphometric evaluation of an indigenous fish belonging to genus Devario from three river systems of South Kerala. *Journal of Biodiversity and Environmental Sciences*, **10**(2), 106-114.

Szlinder-Richert J, Usydus Z, Wyszyński, M, Adamczyk M. (2010). Variation in fat content and fatty-acid composition of the Baltic herring *Clupea harengus membras*. *Journal of Fish Biology*, **77**, 585-599.

Uddin, M. & Okazaki, E. (2004). Classification of fresh and frozen-thawed fish by near-infrared spectroscopy. *Journal of Food Science*, **69**(8). 1750-2788.

Van der Lingen, C.D. (1994). Effect of particle size and concentration on the feeding behavior of adult pilchard, *Sardinops sagax*. *Marine Ecology Progress Series*, **109**, 1-13.

Van der Lingen, C.D. (1999). The feeding ecology of, and carbon and nitrogen budgets for, sardine *Sardinops sagax* in the Southern Benguela upwelling ecosystem. PhD Thesis. University of Cape Town, South Africa

Van der Lingen, C.D., Huggett, J.A. (2003). The role of ichthyoplankton surveys in recruitment research and management of South African anchovy and sardine. In: *The Big Fish Bang Proceedings of the 26th Annual Larval Fish Conference*. Institute of Marine Research (edited by H.I. Browman & A.B. Skiftesvik) Bergen, Norway, pp. 303–343.

Van der Lingen C.D, Freon P., Fairweather T.P, Van der Westhuizen J.J. (2006). Density-dependent changes in reproductive parameters and condition of southern Benguela sardine *Sardinops sagax*. *African Journal of Marine Science*, **28**(3&4), 625-636.

Van der Lingen, C.D., Weston, L.F., Ssempa, N.N. & Reed, C.C. (2014). Incorporating parasite data in population structure studies of South African sardines *Sardinops sagax*. *Parasitology*, **142**, 156-167.

Whitehead, P.J.P. (1985). An annoted and illustrated catalogue of the herrings, Sardines, pilchards, sprats, shads, anchovies and wolf herrings. Part 1, Chirocentridae, Clupeidae and Pristigasteridae. Clupeoid fishes of the world, Vol. 7, FAO species catalogue.UK.

Wold, S., Esbensen, K. & Geladi, P. (1987). Principal component analysis. *Chemometrics and Intelligent Laboratory Systems*, **2**(1-3), 37-52.

Wold, J.P. & Issakson, T. (1997). Non-destructive determination of fat and moisture in whole Atlantic salmon by near infrared spectroscopy. *Journal of Food Science*, **62**(4), 734-736.

Yeannes, M.I. & Almandos, M.E. (2003). Estimation of proximate composition starting from water content. *Journal of Food Composition and Analysis*, **16**, 81-92.

Yeen, T.H., Larivière, S. & Messier, F. (2005), Evaluating body condition of striped skunks using non-invasive morphometric indices and bioelectrical impedance analysis. *Wildlife Society Bulletin,* **33**(1),195-203.

Zhang, H., Zeng, W., Rutman, M. & Lee, T. (2000). Simultaneous determination of moisture, protein, and fat in fish meat using near-infrared spectroscopy. Food Science and Technology Research, **6**(1), 19-23.

Zlatanos, S. & Lastaridis, K. (2007). Seasonal variation in the fatty acid composition of three Mediterranean fish sardine *(Sardina pilchardus*), anchovy (*Engraulis encrasicholus*) and picarel (*Spicara smaris*). *Food Chemistry*, **103**, 725-728.

2 CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

For over 1200 centuries the modern *Homo sapiens* have been consuming marine resources as a food source (Whittle, 2009; Ono, 2016). Interestingly, South African coastal caves inhabited by hunter-gatherers for over 600 centuries show evidence of fish bones, mussel shells (Whittle, 2009). This bears proof to the notion that fish has long been known to be an exceptional source of animal protein and, therefore necessary for a balanced human diet (Marimuthu *et al*., 2012; Strobel *et al*., 2012). Additionally, fish contains vitamins A, B and D and minerals (namely calcium, phosphorous, iodine which are accessible in adequate amounts) and polyunsaturated fatty acids (PUFA) while simultaneously being low in saturated fat (Khan *et al.*, 2016; O'Neil *et al*., 2017).

Apart from fish just being a good source of high-quality protein, vitamins and minerals its consumption is also associated with the reduction of blood pressure and coronary heart disease incidents (Latifa *et al*., 2014; Cheng & Sun, 2015). These health benefits are mainly due to the consumption of fish that contains polyunsaturated long chain omega-3 and omega-6 fatty acids, as well as the essential amino acids, lysine, methionine and cysteine (Tarley *et al*., 2004; Nisa & Asadullah, 2010; Vongsvivut *et al*., 2014).

Data released in 1995 revealed that fish, meat, milk and eggs provided approximately 13.5% of the world's daily protein consumption (Loftas, 1995). According to a more recent study, fish accounted for 15.7% of animal protein and 6.1% of all consumed protein (FAO, 2010). Consequently, global fishing practices have shown a steady increase in the past 50 years, with the fish supply growing at an annual average rate of 3.2% (Anon., 2014a), outperforming world population growth by 1.6% (FAO, 2010). Furthermore, changes in global fish consumption trends over the past 40 years have identified a steady increased consumption of 9.9 kg in the 1960s to 11.5 kg in the 1970s, 12.9 kg in the 1980s and reaching 16.4 kg in 2005, (Anon, 2014a). Also, fish consumption varies significantly among countries due to different consumer preferences, tradition, trade connections, fish availability, product developments, prices and levels of income, to mention a few factors (Anon., 2014a; Muir, 2013). Africa has been indicated to have the lowest consumption of fish with only 9.1 kg per capita (Muir, 2013). Despite the relatively small fish consumption in African countries, fish is seen as one of the essential food components, contributing towards necessary proteins, vitamins and minerals (Gordon *et al*., 2013).

The SA fishery industry comprises of two distinct components, namely wild capture and aquaculture (Anon., 2013b). Aquaculture is a relatively under-utilised component that focuses on high-value marine species such as abalone, mussels and oysters (Anon, 2011; Anon, 2013a). The wild capture component consists of commercial, recreational and subsistence fisheries with commercial captures including small pelagic purse-seine, which includes: sardines (*Sardinops sagax ocellatus*, cape hakes (*Merluccius paradoxus* and *M. capensis*) (deep-water and shallow-water), anchovy (*Engraulis capensis*), round herring (*Entrumeus whiteheadi*) and horse mackerel (*Trachurus capensis*), among others (Anon., 2011; Anon, 2013; Baust *et al*., 2015). Among the pelagic fishing, the SA sardines, are fished and will be further discussed.

2.2 Sardines

The SA sardine (*Sardinops sagax ocellatus*) (Fig. 2.1) is a small pelagic fish belonging to the *Clupeidae* family (Table 1) (Kerstan, 2000; Miller *et al*., 2006). The *Clupeidae* family belongs to the sub-order *Clupeoidei*, which comprises of herrings (*Clupea harengus*), the SA sardine (*Sardinops sagax ocellatus*), sprats (*Sprattus sprattus*), shads (*Alosa sapidissima*), anchovies (*Engraulis encrasicolus*) and wolf-herrings (*Chirocentrus dorab*) (Malseed, 2004). Genetic analysis of the status of sardine (*Sardina*, *Sardinops)* populations, that was conducted by Grant and Bowel (1998) indicates that there are three subspecies of sardinops: *Sardinops sagax ocellatus* from South Africa, Australia and New Zealand, *Sardinops sagax sagax* from the southeastern and northeastern Pacific, and *Sardinops sagax melanostictu*s from the northwest Pacific (Iwamoto & Eschmeyer, 2008). Sardines are omnivorous *Clupeoids*, feeding both on phytoplankton and zooplankton (Van der Lingen, 2002).

Since the 1940s sardines have been a commercially important marine fish species in South Africa's purse-seine fishery, with annual catches peaking at around 400,000 tons in 1962 (Van der Lingen *et al*., 2006; Anon., 2014b). The South African sardine population in the southern Benguela (Fig. 2.2) has undergone substantial rise and fall in size over the past five decades (Van der Lingen *et al*., 2006). An apparent large and healthy population found in the 1950s depleted to low levels in the mid-1960s, where it remained low for 20 more years, only to recover in the late 1980s to a population that is now similar to or larger than that which occurred during the 1950s (Van der Lingen *et al*., 2006).

Figure 2.1: Picture of SA sardine (*Sardinops sagax ocellatus*) covered in a polyethylene bag after capture. Photo was taken using a Huawei P30 Lite

Table 1: Taxon classification of the South African sardine (*Sardinops sagax ocellatus*) (Culley, 1971).

Kingdom	*Animalia*
Phylum	*Chordata*
Class	*Actinopterygii*
Order	*Clupeiformes*
Family	*Clupeidae*
Subfamily	*Incertaesedis*
Genus	*Sardinops*
Species	*Sardinops sagax*
Sub-species	*Sardinops sagax ocellatus*
Common name	The South African sardine (or pilchard)

2.2.1 Distribution of sardine

Figure 2.2 illustrates the distribution of SA sardines off the southern Africa's coastline. It is seen that from the west coast of SA, between St Helena Bay and Cape Agulhas and off the south coast around Mossel Bay and Port Elizabeth, adult sardine is the primary target of the small pelagic

fishery (Hutchings *et al*., 2009; Anon., 2013c). Sardines are also the target of the small seasonal fishing off the South African east coast, where they are caught using beach-seine nets during the KwaZulu-Natal sardine run. The abundance of sardines forms part of the north-easterly migration of fish from the Agulhas Bank to the east coast that occurs almost every winter (Reed *et al*., 2012; Hampton, 2014).

In essence, South African sardines are distributed along the entire South African coastline from the Orange River on the west coast to KwaZulu-Natal (~27°S) on the east coast (Kreiner, *et al*., 2001; Miller *et al*., 2006; Coetzee *et al*., 2008). The variety of the South African sardine extends from southern Namibia where an intense perennial upwelling cell exists and forms a thermal barrier off Luderitz to exchange with the Namibian sardine population, to Richard's Bay on South Africa's northeast coast as illustrated in Fig. 2.2 (Coetzee *et al*., 2008).

Changes in some biological components of the southern Benguela have been observed with sardine showing changes in distribution and location of significant spawning habitat over the past few decades (Van der Lingen, 2002; Van der Lingen & Hutchings, 2005).

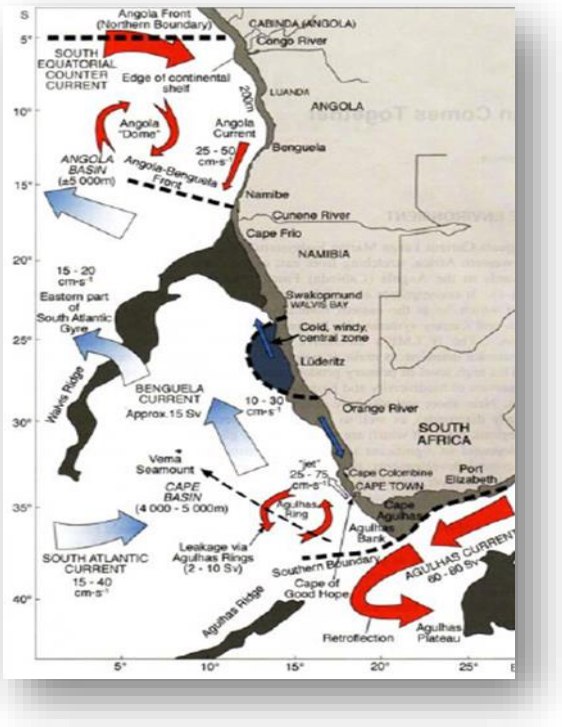


Figure 2.2: A map of Southern Africa showing the distribution of the SA sardines along the western and southern coastlines of South Africa (Coetzee *et al*., 2008).

2.2.2 SA sardine life cycle

Sardines have shown an abrupt shift in spawning between 2000 and 2001 from the west coast to the south coast and have been spawning mainly on the west coast since 2001 (Van der Lingen *et al*., 2006) with some spawning on the west coast in 2007 to 2009 (Mhlongo *et al*., 2015). Spawning of sardines may take place for the first time from the age of two years (Armstrong & Thomas, 1989; Van der Lingen, 1994; Zlatanos & Laskaridis, 2007). Sardine oocytes do not mature instantaneously, and before their sexual maturity, they are known as recruits (Beckley & Van der Lingen, 1999). Female sardines spawn in individual batches on numerous occasions within a single spawning season (Quintanilla & Pérez, 2000). Spawners of this nature are known as batch multiple or serial spawners (Quintanilla & Pérez, 2000).

SA sardines do not have a permanent spawning ground and have been reported to be spawning along almost the entire distributional range in both the west and south coast at different periods (Anders, 1975; Armstrong *et al.,* 1991; Barange *&* Coetzee*,* 1999). Sardines have an extended spawning season that occurs all year round but mainly from August to late March (Anders, 1975). The primary spawning season has two peaks: September to October and February to March (Van der Lingen *et al*., 2002; Van der Lingen & Hugget, 2003). Fowler (1998) concluded that the apparent inconsistency of sardine spawning indicated great flexibility concerning spawning habitat. The flexibility of sardine frees them from being restricted to the Agulhas Bank as their only spawning ground, allowing breeding to extend up the west coast as well (Fowler, 1998). This was later confirmed by Twatwa *et al*. (2005) that sardines are relatively unspecific about their spawning habitat selection and reported that spawning occurs over a broad range of environmental conditions, generally unstable environments which are influenced by strong winds and currents. The primary nursery area for sardines off the South African coastline is between the Orange River and St Helena Bay on the west coast (Armstrong & Thomas, 1989).

2.2.3 Feeding

SA sardines are both particulate and filter feeders (Van der Lingen, 1994,1999). On the west coast, adult sardines feed more on phytoplankton, whereas on the south coast of South Africa they feed more on zooplankton (Low *et al*., 1998; Van der Lingen, 1999). They can capture food particles down to 17 µm in size (Van der Lingen, 1994). Previous studies on the diet of sardines in the Benguela ecosystem showed this species was primarily phytophagous, with a preference for diatoms (Van der Lingen, 2002). Van der Lingen (1994) confirmed that sardines could capture

a considerable amount of phytoplankton through filter feeding and can assimilate diatom carbon-nitrogen (Van der Lingen, 1998). Field studies suggested that filter feeding is the dominant feeding mode used by sardines in its natural environment (Van der Lingen, 2002). The abundance and quality of food in the life cycle of adult sardines is crucial to the condition or fatness of the fish, especially before the spawning season (De Goede, 2004).

2.3 Fish condition

Fish condition refers to the robustness and well-being of fish and is believed to be a good indicator of general fitness of the individual, stock or population under consideration (De Goede, 2004; Sreelekshmi *et al*., 2017). It is an important descriptor of fish health, which can be influenced by fattening, gonad development and stomach fullness (Zorica & Keč, 2012; Lloret *et al*., 2014). Fish condition provides a rough measure of the state of the fish, whether healthy or unhealthy, starved or well-fed, spawning or spent (Patterson, 1992) and may help assess not only the well-being of fish stocks but can also be used as a broad indicator of environmental health (Lloret *et al*., 2014). Fish lipid reserves are an essential characteristic as they have an enormous influence on growth, survival and gonad development (Mustać & Sinovčić, 2009).

Fish condition measures based on weight at a given length and gonad size relative to body size are thought to be reliable indicators of the energetic condition or energy reserves of fish (Lambert & Dutil, 1997). Sardines with high lipid content are considered to be in a better condition than sardines with low lipid content (De Goede, 2004). Poor conditioned fish is usually associated with poor feeding and/or environmental conditions may suffer increased natural mortality (Lambert & Dutil, 2000). Sardine lipid content varies throughout the year (Mustać & Sinovčić, 2009; De Goede, 2004) and it is, therefore, essential for the fish canning industry to have data on lipid content of sardine tissues readily collected (Mustać & Sinovčić, 2009).

Methods used to determine fish condition are time-consuming; such methods include analysis of lipids with gravimetric methods, whereas other methods with lower precision are easy to carry out on board or in the laboratory with minimum equipment and at a low cost (Lloret *et al*., 2014). Ecologists have developed a wide variety of morphological, biological and physiological techniques to assess fish condition (Mustać & Sinovčić, 2009). These methods range from morphological indicators based on length-weight data and morphophysiological indicators based on gonads weight or mesenteric fat (Lloret *et al*., 2014).

2.3.1 Morphological indicators

Morphological indicators are generally considered as simple measures that analyse the variability in length or weight data of fish (Lloret *et al*., 2014; Mojekwu & Anumudu, 2015). Morphological indicators are made from simple weight and length data that can be quickly gathered by non-destructive methods (Vélez-Arellano *et al*., 2016; Murphy *et al*., 1990). Innovative techniques such as ichthyometers and scales for morphometric analysis offer more efficient and powerful tools to identify variances between fish populations, detecting differences among groups and to distinguish between species of similar shape (Mojekwu & Anumudu, 2015). Fish condition indices based on weight and length relationships are easy and inexpensive to acquire, requiring only the fish body length and total weight (Lagrue & Poulin, 2015). Fish condition measurements based on morphometric indicators are non-destructive and can be rapidly obtained in the field and on live fish without the need for laboratory equipment (Lagrue & Poulin, 2015). However, when having to analyse a large number of samples, these methods become time-consuming.

2.3.2 Morphophysiological indicators

Although condition indicators indirectly measure the energetic fitness or well-being of fish based on individual body mass, other condition measures relate directly to the physiological composition of body tissues, providing a more precise measure of the actual fitness regarding stored energy (Lloret *et al*., 2014). Morphophysiological indicators consist of measurements of the ratio between weights of some organs (such as gonads and livers) where energy is stored (Shulman *et al*., 1999; Lloret *et al*., 2014). Among the available morphophysiological indicators, the liver somatic index and mesenteric fat index are the most used. The collection of morphophysiological data is known to be cheap and allows for quick judgement of nutritional condition or status of physiological processes (Vélez-Arellano *et al*., 2016) The assessment of these indicators is time-consuming compared to morphometric indicators because individuals need to be dissected to remove their mesenteric fat (Lloret *et al*., 2014).

2.4 Proximate composition and nutritional importance of fish

The total tissue constituents in fish consist of high-quality protein, fat and fatty acids (in particular the long polyunsaturated marine n-3 fatty acids), vitamins and minerals (Azim *et al*., 2012; Raubenheimer *et al*., 2012; Ayeloja *et al*., 2013), as well as water. Water is the most abundantly

found constituent (up to 80%) in fish (Table 2) (Acharya, 2011). The proximate chemical composition of fish directly relates to the quality thereof (Sutharshiny & Sivashanthini, 2011; Chen *et al*., 2016) and comprises of the moisture, protein, fat and ash contents of the fish flesh (Azim *et al*., 2012). Some carbohydrates are present, but the amount in fish is generally minimal (ca. 0.14%) and is therefore usually not regarded as being significant (Breck, 2014). The knowledge of the proximate composition of fish is vital to estimate their energy value and to plan the most appropriate industrial and commercial processing (Tsegay *et al*., 2016). To food processors, knowledge of fish proximate composition is of great interest because of the effects that various contents of proteins, lipids, water and ash have on the cold storage and texture of fish (Love, 1988).

Table 2: General nutritional composition of fish (Azim *et al*., 2012; Bagthasingh *et al*., 2016)

Nutrient	**Amount (%)**
Moisture	65 – 90
Protein	10 – 22
Fat	0.2 – 25
Ash	0.5 – 5

Biochemical and proximate composition of fish may be affected by both exogenous and endogenous factors (Alemu *et al.*, 2013). Exogenous factors that affect the biochemical structure of fish include fish diet, environmental factors (temperature and salinity of water), fishing time and fishing region (Tang *et al*., 2009; Hantoush *et al*. 2014). Endogenous factors that affect fish composition are linked to genetic factors such as species, body size, sex and age (Alemu *et al.*, 2013). Fish species is the primary factor that influences proximate and fatty acid profile of fish. Fatty acids of animals with single-chambered stomachs are easier to alter than complex-chambered animals (Mraz, 2011). The size of the fish body influences the proximate composition in fish (Breck, 2014). An additional factor with a strong influence on proximate and lipid composition in fish is sex or sexual maturation (Mraz, 2011).

The exogenous factors have insufficient effects compared to the endogenous factors that govern most principles that determine the body composition of fish (Alemu *et al*., 2013). The diet has a significant effect on the proximate and lipid composition of fish ("you are what you eat") (Mraz, 2011). Fish reflects the lipid pattern of its diet to a large extent. The composition and nutritional properties of fish vary with season, maturity and available feed (Bandarra *et al*., 1997).

The protein content of fish varies widely depending on the species. Differences in feeding habits, age, sex of the fish, fat and water content of the flesh cause this variation (Granata *et al*., 2012). The variation of fat at various times throughout the year is another intrinsic factor that can have a profound effect on the quality of fish, in particular, the fatty fish like sardines (Shearer, 1994). Fish is also a rich source of vitamins and minerals (Koubaa *et al*., 2010).

2.4.1 The protein composition of fish

Fish is a vital source of high-quality protein (because of the ideal proportion of essential amino acids) which is easily digestible as it contains less collagen fiber than the protein found in animal proteins (Cheng *et al*., 2013; Sutharshiny & Sivashanthini, 2011; Akpang, 2015; Sumi *et al*., 2016). Proteins are essential in the normal functioning, growth and maintenance of body tissue. Hence, they are regarded as a critical tool to be evaluated for biochemical and physiological standards of a known organism (SK Shahina *et al*., 2016; Ahmed *et al*., 2017). The protein content of seafood generally varies between 11 and 28.4% (usually about 19%) (Venugopal & Shahidi, 2009). After water, protein is an essential component of a fish fillet, with Salmon (*Salmo salar*) muscle, as an example, containing approximately 20 to 22% protein (Savitri, 2011). The contribution of non-protein nitrogenous compounds to the total crude protein content of seafood depends on raw material species and range from 10% to 40% (Venugopal & Shahidi, 2009). The proteins in fish muscle, although being species dependent, can be distinguished as either being (i) myofibrillar, (ii) sarcoplasmic or (iii) stromal (insoluble) (Savitri, 2011). Table 3 shows the average protein content of several fish species.

Table 3: Average protein content (%) of the raw muscle of several fish species

Fish species	Average protein content (%) of fish	Reference
Sardine (*Sardinela longiceps)*	21.29±0.76*	Sumi *et al*. (2016)
Mackerel (*Rastrelliger kanagurta*)	16.75±0.55	
Anchovy (*Stolephorous commersoni*)	16.95±0.27	
Atlantic mackerel (*Scomber scombrus*)	18.50	Olafisoye *et al*. (2013)
Catfish (*Clarias gariepinus*)	11.5	
Tilapia (*Oreochromis niloticus*	16.10	
Roughearscad (*Decapterus tabl*)	22.80	
Snoek (*Thyrsites atun*)	21.5±1.35	Henning & Hoffman (2017)
Tiger shrimp (*Penaeus monodon*)	9.21±0.03	Bernard & Bolatito (2016)
Pink shrimp (*Penaeus notialis*)	6.09±0.05	
Skipjack tuna (*Katsuwonus pelamis*)	24.13±2.01	
Yellowfin tuna (*Thunnus albacares)*	23.52±0.61	Mahaliyana *et al*. (2015)
Bigeye tuna (*Thunnus obesus)*	23.72±0.16	

*mean±SD (Standard deviation)

(i) Myofibrillar proteins

The most significant amount of myofibrillar proteins present in the muscle of aquatic species are myosin, actin, tropomyosin and troponins C, I and T (Fawole *et al*., 2013). Myofibrillar proteins make up 65-75% of the total protein content of these proteins being responsible for the fibrousness, plasticity and gel-forming ability of the fish flesh (Fawole *et al*., 2013). Myosin is the most abundantly found myofibrillar fraction of fish muscles and contributes 50% to 60% of its total protein amount (Rayment & Holden, 1994).

(ii) Sarcoplasmic proteins

The sarcoplasmic proteins, or otherwise known as myogen, comprise mainly of albumin, myoglobin and protein with enzymatic functions (Savitri, 2011). They make up 20 to 30% of the total protein content with the content of sarcoplasmic proteins generally higher in pelagic fish species as compared to demersal fish (Savitri, 2011).

(iii) Stromal proteins

Stromal proteins, although found in small quantities, form the connective tissue of the muscle and comprise of collagen, which is insoluble in neutral salt solutions as well as diluted acids and alkali

solutions (Wang *et al*., 2013). Stromal proteins mount to 3% of the fish muscle (Wang *et al*., 2013).

2.4.2 Lipids and fatty acids associated with fish

Lipids comprise a large and various groups of organic fats and oils that are soluble in organic solvents, but poorly soluble in aqueous solutions (Kennedy *et al*., 2007). Fish are categorised according to the percentage fat in the muscle as either fatty (>10% fat), medium fat (5–10% fat), low fat (2–4% fat) or lean (<2% fat) (Ackman, 1989). Lipids present in the skeletal muscle of fish may be categorised into three major groups namely: (i) phospholipids, (ii) triglycerides (or fatty acids) and (iii) sterols (Kennedy *et al*., 2007).

(i) Phospholipids

Phospholipids play a significant role in the structure of cell membranes as structural lipids (Acharya, 2011). They are esters of fatty acids and phosphatidic acid (Acharya, 2011).

(ii) Triglycerides

Triglycerides, or fatty acids**,** are part of the lipid family with hydrocarbon chains containing a carboxyl group at one end and a methyl group at the other end (Mateos, 2012). Carbon chains of different length indicate the degree of saturation (Trattner, 2009; Çibuk *et al*., 2014). Fatty acids can further be categorised into three groups namely: (a) saturated (SFA), (b) monounsaturated (MUFA) and (c) polyunsaturated (PUFA) fatty acids, based on the length of the respective carbon chain (Mateos, 2012). Therefore, fatty acids with less than eight carbons are seen as being short-chain fatty acids, those with eight to fourteen carbons are categorised as medium chain fatty acids and the fatty acids with sixteen to twenty-two, or more, carbons are seen as long chain fatty acids (Rustan & Drevon, 2005; Mateos, 2012).

(iii) Sterols

Sterols are subgroups of steroids that naturally occur in both plant and animal species (Gordon, 2003; Rustan & Drevon, 2005). Sterols play an essential role in the membrane integrity of eukaryotes where they control biological processes and sustain the domain structure of cell membranes (Dufour, 2008; Weatherby & Carter, 2013). The most commonly known sterol in human is the cholesterol. Cholesterol in foods occurs as either free alcoholic sterol or as cholesteryl esters (Fenton, 1992; Toivo *et al*., 2001). *Saturated fatty acids* (SFAs)*:* Fatty acids with no double bond in their chain length are referred to as saturated fatty acids (Mraz, 2011). The most common saturated fatty acids are palmitic acids (PA C16:0), lauric acid (LA C12:0), myristic

acid (MA C14:0) and stearic acid (SA C18:0) (Sekar *et al*., 2017). Saturated fatty acids with less than eight carbon atoms are liquid at room temperature, whereas those containing more than eight are solid at room temperature. Figure 2.3 illustrates the chemical structure of the two most common saturated fatty acids.

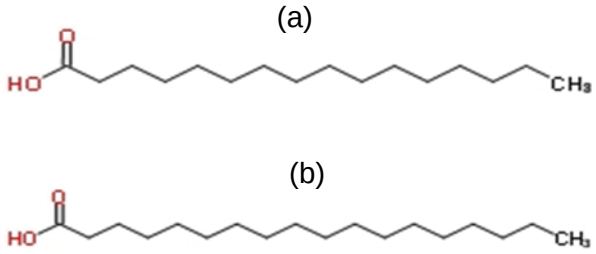


Figure 2.3: Chemical structures of (a) palmitic and (b) stearic acid (Rustan & Drevon, 2005).

Monounsaturated (MUFAs) and polyunsaturated (PUFAs) fatty acids: Unsaturated fatty acids comprise one or more double bonds in their carbon chain. Fatty acids are further categorised based on the number of double bonds present (Mraz, 2011). Those with one double bond are referred to as monounsaturated while those with two or more double bonds are known as polyunsaturated fatty acids (Kennedy *et al*., 2007; Rustan & Drevon, 2005).

Fish is known to be a rich source of omega-3 PUFA especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Gamez-Meza *et al*., 1999; Cascant *et al*., 2018). Omega-3 PUFAs come in a variety of short and long chain molecules (Mraz, 2011). Alpha-linoleic acid (ALA, 18:3n-3) with eighteen carbons and three double bonds molecules is the short structure of the omega-3 PUFA (Lau, 2007), while docosahexaenoic, 22:6n3 (DHA), docosapentaenoic acid, 22:5n-3 (DPA) and eicosapentaenoic acid, 20:5n-3 (EPA) are long chain fatty acids (Lau, 2007).

In their natural state, unsaturated fatty acids are in the form of omega-9, omega-6 and omega-3 and these are individually known as oleic, linoleic and linolenic acids (Çibuk *et al*., 2014). Omega-3 and omega-6 PUFA (also referred to as n-3 and n-6) are common PUFAs (Mraz, 2011). A healthy diet should consist of a balanced n-6:n-3 ratio (Bhardwaj *et al*., 2016). The dietary balance of n-6 and n-3 fatty acids, which both can be obtained in the diet, is essential for homeostasis and healthy development in humans (Simopoulos, 2000; Totsch *et al*., 2015).

Generally, it is beneficial that the ratio of omega-6 to omega-3 fatty acids is close to 4:1 in the diet (Totsch *et al*., 2015).

The amount of n-3 to eat depends on how much n-6 consumers eat (Bhardwaj *et al*., 2016) . Reducing dietary n-6 fatty acid (i.e. linoleic acid) intake increases the availability of n-3 fatty acids which may lower tissue concentrations of the n-6:n-3 ratio, reducing the intensity and duration of inflammatory responses and consequently reduce the risk of the disease (Johnson & Bradford, 2014). It has long been known that the amount of n-6 in the diet affects the conversion of n-3 ALA (α-linolenic acid) to long-chain n-3 EPA and DHA which protect humans from diseases (Bhardwaj *et al*., 2016).

Unlike saturated fatty acids, n-3 fatty acids have been associated with health benefits relating to the treatment of rheumatoid arthritis (Gogus & Smith, 2010). Dietary intake of n-3 fatty acids influences the expression of several genes such as peroxisome proliferator-activated receptors (PPARs) and sterol regulatory element binding protein (SREBP) (Price *et al.*, 2000). Additionally, omega-3 fatty acids are associated with the synthesis of eicosanoids, thromboxanes and leukotrienes (Rustan & Drevon, 2005). Moreover, these omega-3 fatty acids cannot be synthesised in the human body and have to be supplemented from the diet (DeLorgeril *et al.*, 2001; Chauke *et al*., 2008; Jabeen & Chaudhry, 2011). Therefore, the importance of consuming fish cannot be neglected (Çelik *et al.*, 2005*;* Zlatanos and Laskaridis, 2007; Bagthasingh *et al.*, 2016).

Fish is an exceptional source of lipids with high PUFA content (Bandarra *et al*., 1997), especially EPA (C-20:5) and DHA (C-22:6) are of particular interest because of their role in improving human health. EPA has been reported to be useful in cancer treatment and brain disorders, whereas DHA is a significant component which plays a fundamental role in brain and eye development (Tang *et al*., 2009). Although the best source of long-chain n-3 PUFA is fish and fish products, fat content and fatty acid profiles change with species, season and diet. Sardine species such as *Sardinops sagax ocellatus*, *Sardina pilchardus* and *Sardinops melanostictus* in temperate regions show seasonally fluctuating in fatty acid composition and yield, influenced by seawater temperature, food availability and sexual state of the animal (Chitra Som & Radhakrishnan, 2013). Fish fatty acids are not only nutritionally essential; they play a fundamental role in the development of the gonads, are transferred to ovaries and egg constituents during reproduction (Mika *et al*., 2012). In marine species, the PUFA, 22:6n-3 is essential for the growth and survival of these species as well as being the main component of the neural tissues.

2.5 Conventional methods for the determination of fish constituents

2.5.1 Protein content

Protein analysis methods date back to the 18th century (Moore *et al*., 2010). The total protein composition of any foodstuff is essential in ensuring the requirements of food regulations and commercial specifications, safety and quality as well as trade are complied with (Sutharshiny & Sivashanthini, 2011; Moore *et al*., 2010). The two methods primarily used for protein analysis are (i) the Kjeldahl method and (ii) the Dumas combustion method, e.g. the LECO protein analyser (LECO Corporation, Saint Joseph, Michigan, USA). These two methods depend on the determination of nitrogen as an indicator to estimate protein content (Moore *et al*., 2010).

(i) Kjeldahl method:

The Kjeldahl method is most frequently used because of its robustness, simplicity and accuracy (Conklin-Brittain *et al*., 1999). The Kjeldahl method is based on the digestion of ground sample in a concentrated sulphuric acid solution, which contains a catalyst such as selenium or mercury (Da Silva *et al*., 2016). The method involves three steps, namely:

(a) digestion, where the sample is digested in strong sulphuric acid solution in the presence of a catalyst that helps in the conversion of the amine nitrogen to ammonium ions (Perrson, 2008; da Silva *et al*., 2016);

(b) distillation, where the ammonium ions are then converted into ammonia gas, heated and distilled. The ammonia gas is led into a trapping solution where it dissolves and becomes an ammonium ion once again (Perrson, 2008; Da Silva et al., 2016); and lastly

(c) titration, where the amount of the ammonia that has been trapped is determined by titration with a standard solution and appropriate calculations are made (Perrson, 2008).

(ii) Dumas combustion by LECO protein analyser:

The Dumas combustion method is a method used to determine the total nitrogen content in an organic matrix (Müller, 2014). With the innovative improvement in dry combustion nitrogen analyser technology and with the costs involved in the disposal of hazardous chemicals, the Dumas method is replacing the traditionally used Kjeldahl method for nitrogen analysis (Zaldivar, 1998; Sader *et al*., 2004). The LECO system involves:

(a) total combustion of the sample at a high temperature (900 – 1200°C) in the presence of oxygen, whereby carbon and nitrogen are converted to carbon dioxide and NO_x (Sader *et al*., 2004; Muller, 2014);

(b) passing the gases over unique columns that absorb carbon dioxide and water (Sader *et al*., 2004; Müller, 2014);

(c) separating the nitrogen from any remaining carbon dioxide and water using a column that contains a thermal conductivity detector (Sader *et al*., 2004); and measuring the residual nitrogen content.

2.5.2 Lipids and fatty acid content

Various lipid extraction methods are applicable for measuring fats in fish (Min & Ellefson, 2010). The accurate determination of lipids in foods is necessary for nutritional labelling, certification as well as to determine their influence on the nutritional properties of foods (Decker, 2005). Amongst others, these methods include the quantification of lipids using (i) solid-liquid extraction in Soxhlet apparatus, (ii) 1-step solvent extraction method, and (iii) 3-step solvent extraction.

(i) Soxhlet extraction method:

The Soxhlet extraction method involves a constant flow of organic solvent such as petroleum ether over the sample. The solvent is boiled, condenses and passes the sample, thereby extracting the lipids. After a sufficient time, the process is stopped, the solvent is evaporated, and fat is weighed (Lau *et al*., 2010).

(ii) 1-Step extraction method:

Lipid quantification using the 1-step extraction method involves the solvent extraction using a mixture of water and chloroform-methanol (2:1), followed by a washing step with KCl (Folch *et al*., 1957).

(iii) 3-Step extraction method:

The determination of lipids using the 3-step extraction method firstly the use of a methanol and chloroform solution, whereas the second step makes use of only chloroform and at the last step water is added to the sample. Using gravimetric analysis, following evaporation of the solvent, the total lipid content is determined (Bligh & Dyer, 1959).

2.6 Near-infrared (NIR) spectroscopy

2.6.1 Introduction to near-infrared (NIR) spectroscopy

The use of near-infrared (NIR) energy was first discovered in 1800 by William Frederick Herschel, a German-born, English scientist (Herschel, 1800; Williams, 2009). He observed the first NIR spectrum while evaluating the heat emitted by the solar energy beyond the red portion of the visible spectrum (Pasquini, 2003; Williams, 2009; Nawrocka & Lamorska, 2013).

NIR spectroscopy is based on the electromagnetic absorption of organic compounds at wavelengths between 780 to 2500 nm (14300 to 4000 cm^{-1}) (Fig. 2.4) (Osborne *et al*., 1993; Pasquini, C., 2003). However, the wavelength region between 1200 and 2500 nm (8333 to 4000 cm^{-1}) are generally most frequently used for quantitative analysis due to the adequate strength of the absorption bands in this region (Norris, 1989). From the latter statement, it is thus evident that the NIR region contains information relating to relative proportions of bands, which vary according to the structural components of organic molecules (Cozzolino *et al*., 2001; Cozzolino *et al*., 2004).

When spectral data is collected, NIR radiation interacts with the sample and a portion of the radiated energy may be absorbed, transmitted or reflected (Osborne, 1993; Pasquini, C, 2003; Huang *et al*. 2008; Bag *et al*., 2011; Prieto *et al*., 2017). Different modes of measurements can be applied in NIR spectroscopy for different applications. These measurement modes include transmittance, transflectance, diffuse reflectance, diffuse transmittance and diffuse transflectance (Osborne *et al*., 1993; Pasquini, 2003; Prieto *et al*., 2017). The choice of measurement mode depends on sample characteristics, for example the phase (i.e solid or liquid) and on particle size (Prieto *et al*., 2017).

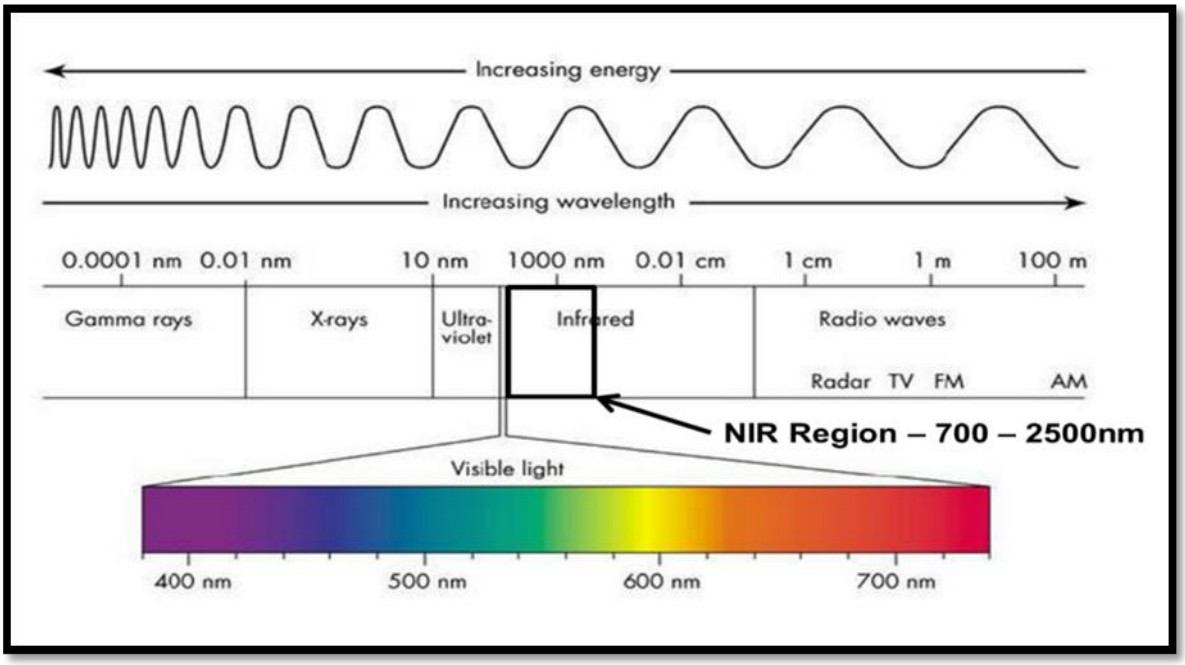


Figure 2.4: Position of NIR region in the electromagnetic spectrum (Brown, 2013).

Spectroscopy deals with the absorption and emission of light by matter as a function of the wavelength of the radiation (Njuguna *et al*., 2015). The absorption of light is explained by the Beer-Lambert law, which states that when a compound that absorbs light is dissolved in a non-absorbing medium, the attenuation is proportional to the concentration of the compound in the solution and the optical path length (Bakker *et al*., 2012):

$A = \log_{10} [I_o/I] = a \times c \times d$ Equation 2.1

Where $A = a \times c \times d$

Absorbance (A) is the logarithmic ratio of two intensities, i.e. the intensity of the incidence light (I_o) and the transmittance light (I); and

a = specific coefficient of the absorbing compound

c = concentration of the absorbing compound in the solution

d= the distance between the points where light enters and leaves the medium.

In this NIR region, overtones and vibrational modes involving C-H (fats, oils, hydrocarbons), O-H (water, alcohol), N-H (protein) and possibly S-H and C=O chemical bonds overlap (Osborne *et al*., 1993; Nawrocka & Lamorska, 2013). These chemical bonds (C-H, O-H, N-H, S-H and C=O) are subjected to vibrational energy changes when irradiated by NIR frequencies, resulting in absorption bands (Osborne *et al*., 1993) (Fig. 2.5).

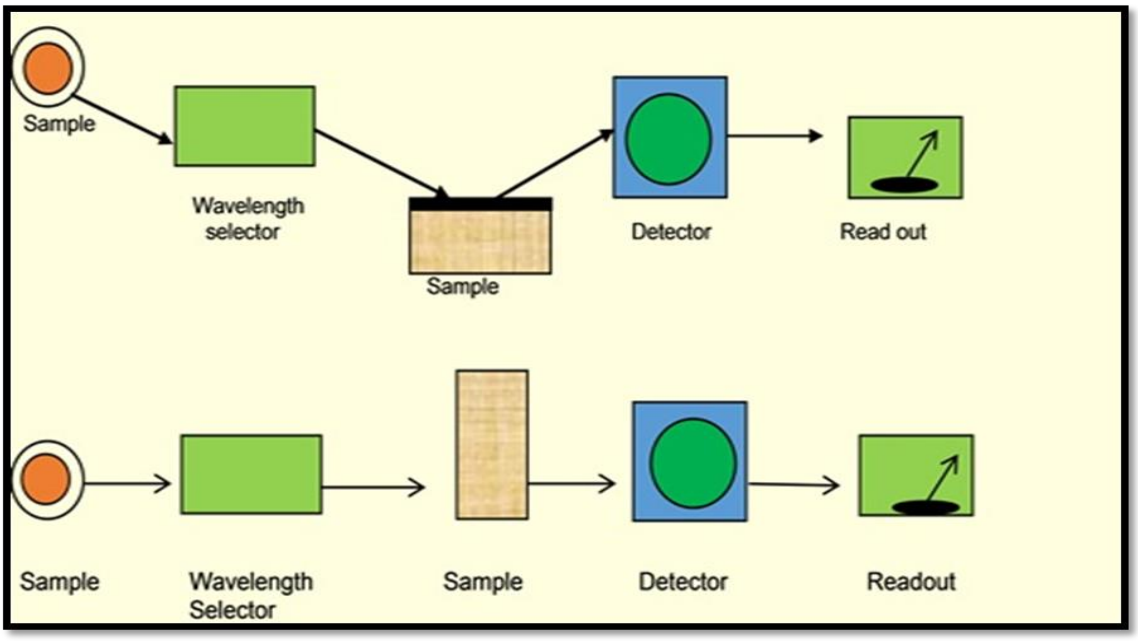


Figure 2.5: Basic configurations for (a) reflectance and (b) transmittance, which are the two commonly used NIR spectroscopy measurement approaches.

2.6.2 Near-infrared (NIR) instrumentation

The primary components of a NIR instrument consist of, amongst others, a (i) light source, a (ii) wavelength selector (or a monochromator) and a (iii) detector (Wehling, 1994; Wetzel 1998).

(i) The light source is necessary for continuously emitting radiation in the NIR wavelength range (Challadurai & Jayas, 2014). Tungsten-halogen lamps with quartz windows are most commonly used as light sources due to their longer life and better stability as well as their capability to emit a continuous spectrum (Challadurai & Jayas, 2014; Prajapati *et al.,* 2016).

(ii) The wavelength selector or monochromator is an essential component of a NIR instrument as it facilitates the dispersion of the radiation according to the required wavelengths (Challadurai & Jayas, 2014). Prisms and grating devices are the two frequently used dispersing instruments (Challadurai & Jayas, 2014).

(iii) The radiation from the light source dispersed by the monochromator to a sample absorbs some radiation and reflects or transmits the remaining radiation (Challadurai & Jayas, 2014). The reflected or transmitted radiation is then measured by the detector (Challadurai & Jayas, 2014). Detectors can be categorised into two groups, i.e. thermal and photon detectors (Challadurai & Jayas, 2014). The main difference between the thermal and photodetectors is the dependency of defectiveness of wavelength and temperature (Rogalski, 2002). Incident radiation is absorbed to change the temperature of the material in thermal detectors, while in photon detectors it is incorporated within the material through the interaction with electrons. Thermal detectors are generally ideal at wide wavelength ranges, whereas photon detectors are preferred at shorter wavelength ranges and lower operating temperatures.

Several NIR spectroscopy measurement approaches are available depending on the respective applications. In general, the two methods most used, are transmittance and reflectance (Fig. 2.5) (Osborne *et al*., 1993; Berzaghi & Riovanto, 2009). The apparent difference between the two approaches involves the recording of the portion of light not absorbed by the matter when transmitted through the sample, as referred to as transmittance, whereas reflectance records light bounced off the sample. For the latter, the detector is situated above or below the sample, whereas with the transmittance set-up the detector is located behind the sample (Cen & He, 2007; Khodabux *et al*., 2007).

Various types of NIR instruments exist, which include scanning, Fourier Transform, acoustic optical tunable filter, as well as photodiode array spectrophotometers (Aenugu *et al*., 2011). Of late, portable hand-held NIR instruments have become available, which will be discussed in the following section.

2.6.3 Hand-held NIR spectrophotometers

There are many studies underlining the potential of NIR spectroscopy on fish and fishery products and endorsing its use as a non-destructive rapid analytical technique used for determining quantitative or qualitative characteristics (Malegori *et al*., 2017). NIR spectroscopy has increasingly developed into an essential analytical tool for quality control in many industries, i.e. the manufacturing industry (Blanco *et al*., 1998; Pasquini, 2003). However, conventional NIR instruments are bench-top laboratory-based instruments. Usually, most analyses are performed by taking samples from the production site and transporting it to the quality control laboratory that

is separately situated (Alcala *et al*., 2013; Dérgadin *et al*., 2017). Consequently, there is a delay between sampling and getting the results, which limits the regularity of analysis.

Recently, considerable attention has been given to the development of miniaturised, precise portable NIR spectrophotometers capable of performing rapid, at line or inline analysis, as these are seen as practical quality control tools (Alcala *et al*., 2013; Santos et al., 2013; Haughey *et al*. 2015; Malegori *et al*., 2017; Pezzei *et al*.,2017). The miniaturisation of spectrometers began approximately fifteen years ago, but only within the last few years have the real handheld Raman, infrared (IR), and NIR scanning spectrophotometers become commercially available (O'Brien *et al*., 2012).

NIR technology is offered in large benchtop instruments or portable, lightweight instruments that can be carried in a backpack to the field (Davies, 1998; Dérgadin *et al*., 2017). These portable devices operate using the same mechanisms and principles as benchtop instruments but offer unique properties. The improved, lightweight handheld instrument does not require sample preparation (drying and grinding) before analysis (Guo *et al*., 2010). Portable NIR devices offer several advantages for nondestructive, non-hazardous online analysis: small size, low cost, robustness, the simplicity of analysis, portability, and environmentally friendly (Dérgadin *et al*., 2017). An example of such a portable NIR device (Fig. 2.7a), is Viavi's MicroNIR spectrophotometer (Viavi Solutions Inc., Milpitas, CA, USA).

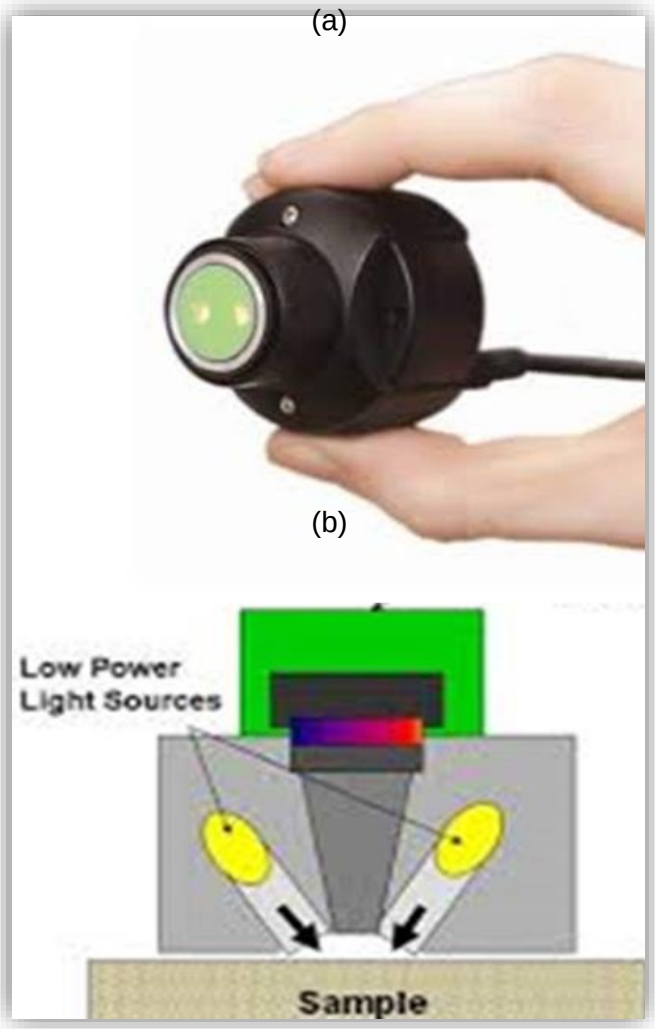


Figure 2.6: An ultra-compact version of a NIR spectrophotometer is presented in the form of (a) the MicroNIR, with (b) a simplified illustration of the respective instrument (Alcala *et al.,* 2013).

The instrument differs from the conventional NIR spectrophotometers in that it is significantly smaller (Zumba & Rodgers, 2016; Guelpa, 2015; O'Brien *et al*., 2013). It is an ultra-compact, hand-held version of the NIR spectrophotometer, weighing less than 60 g and measures < 50 mm (O'Brien *et al*., 2013). The instrument covers the wavelength range of 887 to 1667 nm (O'Brien *et al*., 2013). Contained in the small device is a light source (2 lamps), a dispersing element, a detector and electronics (Fig. 2.6b) (O'Brien *et al*., 2013). A significant advantage of the MicroNIR is the ability of the device to fit in the pocket of a food inspector, and as an example, to be used at the receiving dock of a seafood plant (O'Brien *et al*., 2013). The small size of the MicroNIR is

due to the new thin-film linear variable filter (LVF) that serves as a dispersive element (Alcala *et al*., 2013). The LVF is a dielectric thin-film Fabry-Perot bandpass filter element that is deposited using dynamic processes (Alcala *et al*., 2013; Pederson *et al*., 2014). The LVF is positioned over a diode array detector that scatters light into individual wavelengths (Anon., 2014c).

2.6.4 Near-infrared (NIR) spectroscopy as an alternative to conventional methods

Spectroscopic methods have been historically very successful at evaluating the quality of agricultural products such as meat, fish and fishery products, fruits and vegetables, grain, dairy products, and also in other areas such as pharmaceuticals (Huang *et al*., 2008; Nawrocka & Lamorska, 2013). NIR spectroscopy is a method nowadays routinely used for fast and non-destructive measurements of compositional, functional and sensory quality attributes of food products, amongst others (Karoui & De Baerdemaeker, 2007). It has become an alternative quality control method to the conventionally used chemical and sensory methods (Karlsdottir *et al*., 2014). The respective NIR spectroscopic methods are highly desirable for analysis of food components because they often require minimal or no sample preparation, provide rapid, and on-line analysis, and have the potential to run multiple tests on a single sample (Saeed *et al*., 2012; Olivos-Trujillo *et al*., 2015). Some applications of NIR spectroscopy include the assessment of fat, moisture and protein in emulsified or ground meats (Uddin *et al*., 2002), as well as the measurement of protein, moisture, ash and kernel hardness as applied in the maize industry (Manley *et al*., 2013).

Various spectroscopic techniques including NIR, Raman, nuclear magnetic resonance (NMR) and spectral imaging have been developed efficiently for fish analysis, with the last three being costly and not easy to use for online detection (Hernandez-Martinez *et al*., 2014). Therefore, NIR spectroscopy has been successfully applied on fish-related studies as indicated by some comprehensive reviews (Weeranantanaphan *et al*., 2011; Cozzolino & Murray, 2012; Liu *et al*., 2013; Cheng *et al*., Cheng *et al.* 2014a, 2014b) published since 2011 and some more studies are summarised in Table 4.

Table 4: Some studies related to NIR spectroscopic application on fish and fish products

Study purpose	Sample	Quality parameters	References
To predict *Pseudomona*s counts	Salmon fillets	*Pseudomonas* counts	He & Sun (2015)
To assess texture and colour	Rainbow trout	Texture & colour	Zotte *et al*. (2015)
To predict the proximate chemical composition and identify the rearing system	European sea bass	Moisture, protein, ether extract and gross energy	Xiccato *et al*. (2014)
To assess freshness	Atlantic salmon	Sensory & Microbiological analysis	Kimiya *et al*. (2013)
To monitor time and temperature history	Hake fillets	Lipids	Careche *et al*. (2015
To examine endpoint temperature	Fish and shellfish	Endpoint temperature	Uddin *et al*. (2002)
To differentiate between frozen and thawed	Prawns	Total olatile Basic Nitrogen (TVB-N)	Dai *et al*. (2015)
To quantify proximate composition	Tuna	Proximate composition	Khodabux *et al*. (2007)
To determine protein	Herring	Protein content	Svensson *et al*. (2004)
To predict the proximate composition	Fishmeal	Moisture & Crude protein	Saeed *et al*. (2012)
To identify fish species	Mackarel, herring, salmon, blue whiting	Proximate composition, TVN	Cozzolino *et al*. (2005)

2.7 Multivariate data analysis (MDA)

Multivariate data analysis (MDA) provides a powerful tool for the extraction of relevant chemical information from generated spectra that would correlate with the measured component (e.g proximate composition) under investigation (Cozzolino *et al*., 2006; Wang & Mizaikoff, 2008; Manley, 2013; Manley 2014). The greatest benefit of MDA is the ability to reduce the amount of data, build classification models as well as improve the accuracy and robustness of models (Kucha

et al., 2018). Among the most commonly used MDA methods, are principal component analysis (PCA) and partial least squares (PLS) regression (Mobley *et al*., 1996; Yang *et al*., 2003; Wiesner *et al*., 2014). If the relation of context contains large variation, then PCA is a good exploratory method (Pearson, 1901; Wold *et al*., 1987; Hastie *et al*., 2009).

2.7.1 Principal component analysis (PCA)

PCA is an unsupervised method among the MDA techniques that are normally used in exploring data (Martens & Naes, 1989; Pere-Trepad *et al*., 2006). PCA is used for the reduction of data and thereby permits the interpretation of large datasets (Li *et al*., 2010; Šimat *et al*., 2012). PCA involves the transformation and visualisation of complex data sets as it converts the data into new simpler or smaller sets called principal components (PCs) (Massart, 1998). The first PC explains the most important variation in the data set, or the variation that represents the biggest percentage of the data, whereas the second PC explains the second most important variation/percentage (Esbensen, 2002). Therefore, the more relevant information can be easily obtained when data is decomposed into PCs as explained by Equation 2.2. (Flaete *et al*., 2006; Pere-Trepad *et al*., 2006):

$$X = T_a P_a^T + E \qquad \text{Equation 2.2}$$

where X is the spectral data, *a* is the number of PCs, T represents the scores and P the loadings. The scores (T) provide the coordinates of the samples in the PC space and allow the similarity/dissimilarity of the sample to be inspected (Esbensen, 2002). Additionally, the loadings (P) characterise the weight with which each original variable contributes to the PCs, allowing the correlation structure among the variables through the loading scatter plots to be inspected (Massart, 1998). Represented by E is the residual which is part of the original data not described by the model (i.e. noise) and is used to indicate outlying samples/variables (Massart, 1998; Li Vigni *et al*., 2013).

2.7.2 Partial least squares (PLS) regression

Unlike PCA models that are seen as exploration models, PLS regression models are constructed to perform predictions. When describing variables to be modelled in a regression context, the Y-variable is seen as the dependent variable (i.e. the wavelengths when using NIR spectroscopic generated data) and the X-variable as the independent variable (predictor, i.e. a chemical constituent like protein content) (Westad *et al*., 2013). PLS is a linear modelling technique that compresses the spectral data and projects it on to PLS components (Flaete *et al*., 2006; He &

Sun, 2015). PLS computations are customarily performed automatically, calculating the variables in the order of importance (only the useful number of PCs or latent variables are kept in the model) (Osborne *et al*., 1993).

To evaluate the success of a prediction model, the sample set is split into a training set (used for model building) and a validation set that is subjected to a cross-validation process. Several statistical parameters may express the robustness or goodness of the prediction of the methods, and one of the most used is the root mean square error of cross-validation (REMSECV) or root mean square error of prediction (REMSEP) dedpending on the type of prediction undertaken (Naes & Isaksson, 1991; Prieto *et al*., 2017). An additional measure of the goodness of the prediction model is the (iii) ratio performance deviation (RPD), which incorporates the standard deviation (SD) associated with the reference method used in the calibration (Cozzolino *et al*., 2006).

(i) RMSECV (Eq. 2.3) expresses the goodness of the model as the root mean square error of cross-validation (RMSECV) when cross-validation is performed (Fulop *et al*., 2008). The more RMSECV converges to zero, the better the model (Fulop *et al*., 2008). RMSECV is an estimation of error from a form of validation (Wehrens, 2011).

$$\text{RMSECV}=\sqrt{\frac{\sum_{i=1}^{n}(y_1-\dot{\hat{y}}_1)^2}{n}} \qquad \text{Equation 2.3}$$

Where y_i = the reference value for the i^{th} sample

$\hat{y}$ = the predicted value for the i^{th} sample when it was dropped from the regression

n = the number of samples

(ii) RMSEP (Eq. 2.4) is the direct estimation of the prediction and modelling errors of the calibration against the reference method using an independent test set (Naes & Issaksson, 1991; Esbensen, 2008).

$$\text{RMSEP}=\sqrt{\frac{\sum_{i=1}^{n}(y_1-\dot{\hat{y}}_1)^2}{n}} \qquad \text{Equation 2.4}$$

Where y_i = the reference value for the i^{th} sample

$\hat{y}$ = the predicted value for the i^{th} sample

n = the number of samples

The ratio of performance to deviation (RPD) (Eq. 2.5) is the relationship between the SD of the population (it refers to the error associated with the specific reference method, i.e. LECO protein method) divided by the standard error of prediction (SEP). A high RPD value is desired as it indicates the effectiveness of a calibration model as can be interpreted in Table 5 (Williams, 1991). In other words, if a high RPD value for a prediction model is obtained, the error associated with the model is very similar to the error related to the reference method. The NIR prediction model can, therefore, replace the reference method.

$$\mathrm{RPD} = \frac{\mathrm{SD}}{\mathrm{SEP}} \qquad \text{Equation 2.5}$$

Where SD = the standard deviation

SEC = the standard error of prediction

Table 5: Interpretation of RPD values (Williams, 1991)

RPD value	Prediction	Application
0.0 - 2.3	Not recommended	-
2.4 – 3.0	Poor	Very rough screening
3.1 – 4.9	Fair	Screening
5.0 – 6.4	Good	Quality control
6.5 – 8.0	Very good	Process control
8.1+	Excellent	Any application

2.8 Conclusion

Fish and fishery products are still regarded as the most important part of human diet. The quality of fish can be evaluated using a variety of traditional methods. However, there is a need for fast, non-destructive techniques for quality evaluation and stock assessment. The application of NIR spectroscopy in fish has increased as this technique has the advantages of being rapidly applied with no sample preparation. Many studies involving NIR have shown this technique to be a powerful analytical tool suited for quantitative analysis of quality attributes in both homogenized

and intact fish muscle, and therefore has the potential to replace conventional chemical methods in quality control methods. NIR spectroscopy could be used as a successful technology for online monitoring of fish processing. The portable miniature MicroNIR offers fast, non-destructive and non-hazardous techniques that can be carried out on-site (i.e. receiving dock of a fish processing plant). The use of NIR spectroscopy as a non-invasive, rapid, environmentally friendly analytical technique might be substantive for fish processors and would need further exploration for online application.

References

Ackman, R.G. (1989). Nutritional composition of fats in seafoods. *Progress in Food & Nutrition Science*, **13**(3-4), 161-289.

Aenugu, H.P.R., Kumar, D.S., Srisudharson, Parthiban, N., Ghosh, S.S. & Banji, D. (2011). Near infrared spectroscopy- an overview. *International Journal of ChemTech Research*, **3**(2), 825-836.

Ahmed, E.O., Ahmed, A.M., Ebrahim, S.J. & Adm, H.H. (2017). Proximate and mineral composition of some commercially important fishes in Jebl Awlia reservoir, Sudan. *International Journal of Aquaculture Research*, **3**(1), 51-57.

Akpang, V.O.E. (2015). Proximate composition of some tropical fish species. *Der Chemica Sinica*, **6**(4), 125-129.

Alcala, M., Blanco, M., Moyano, D., Broad, N. W., O'Brien, N., Friedrich, D., Pfeifer, F. & Siesler, H. W. (2013). Qualitative and quantitative pharmaceutical analysis with a novel hand-held miniature near infrared spectrometer. *Journal of Near Infrared Spectroscopy*, **21**, 445-457.

Alemu, L.A., Melese, A.Y. & Gulelat, D.H. (2013). Effect of endogenous factors on proximate composition of Nile tilapia (*Oreochromis niloticus L.)* fillet from lake Zeway. *American Journal of Research Communication*, **1**(11), 405–410.

Anders, A.S. (1975). Pilchard and anchovy spawning along the Cape east coast. *South African Shipping News and Fishing Industry Review*, 53-57.

Anonymous, (2014a). Status and trends. *World Review of Fisheries and Aquaculture, Part 1*. [WWW document]. URL http://www.fao.org/3/a-i3720e/i3720e01.pdf . Accessed 26 April 2017.

Anonymous, (2014b). Small pelagic fish (sardine, anchovy and round herring). In: *Status of the South African Marine Fishery Resources 2014*. Department of Agriculture, Forestry and Fisheries. Cape Town, South Africa.

Anonymous, (2014c). Fit for purpose MicroNIR spectrometer facilitates quality by designadoption.[WWWdocument].URLhttp://www.jdsu.com/ProductLiterature/micronirpro-ds-co-ae.pdf. 28 May 2015.

Anonymous, (2013a). Fish to 2030: Prospect for Fisheries and Aquaculture. *The World Bank Report 83177-GLB*, Washington, USA.

Anonymous, (2013b). Fishery Accounts for South Africa: 1990-2011. (D0405.0). Pp. 2-3. Statistics South Africa.

Anonymous, (2013c). Status and management of South African small pelagic fishery.[WWWdocument].URLhttp://oceana.co.za/wpcontent/uploads/2016/09/Status and management of the South African small pelagic fishery 2013.pdf. 28 November 2016.

Anonymous, (2011). South Africa's Sectors. In: *Fisheries: Facts and Trends in South Africa.* World Wide Fund. p. 9

Archarya, D. (2011). Fillet quality and yield of farmed Atlantic salmon (*Salmo salar* L): Variation between families, gender differences and the importance of maturation. MSc Thesis, Norwegian University of Life Sciences, Norway.

Armstrong, M. J. & Thomas, R.M. (1989). Clupeoids. In: *Oceans of life* (edited by A. I .L Payne & R. J. M Crawford). Pp. 105-121. Publishers: Vlaeberg. Cape Town.

Armstrong, M.J., Chapman, P., Dudley, S.F.J., Hampton, I. & Malan, P.E. (1991). Occurrence and population structure of pilchard *Sardinops ocellatus*, round herring *Etrumeus whiteheadi* and anchovy *Engraulis capensis* off the east coast of southern Africa. *South African Journal of Marine* Science, **11**, 227-249.

Ayeloja, A.A., George, F.O.A., Dauda, T.O., Jimoh, W.A. & Popoola, M.A. (2013). Nutritional comparison of captured *Clarias gariepinus* and *Oreochromis niloticus*. *International Journal Research Journal of Natural Sciences*, **1**(1), 9-13.

Azim, M.A., Islam, M.R., Hossain, M.B. & Minar, M.H. (2012). Seasonal variations in proximate composition of gagentic Sillago, Sillagnopsis panyus (*Perciformes*: *Sillagnidae*). *Middle-East Journal of Scientific Research,* **11**(5), 559-562.

Bag, S.K., Srivastav, P.P. & Mishra, H.N. (2011). FT-NIR spectroscopy: a rapid method for estimation of moisture content in bael pupl. *British Food Journal*, **113**(4), 494-504.

Bagthasingh, C., Aran, S.S., Vetri, V. & Innocen, A. (2016). Seasonal variation in the proximate composition of sardine (*Sardinella gibbosa*) from Thoothukudi coast*. Indian Journal of Geo-Marine Science*, **45**, 800-806.

Bakker, A., Smith, B., Ainslie, P. & Smith, K. (2012). Near-infrared spectroscopy applied*. Aspects of Ultrasonography in Humans*, **190**, 65-88.

Bandarra, M.N., Batista, I., Nunes, M.L., Empis, J.M. & Christie, W.W. (1997). Seasonal changes in lipid composition of sardine (*Sardina pilchardus*). *Journal of Food Science*. **62**(1), 40-42.

Barange, M. & Coetzee, J. (1999). Results of the 1999 pelagic recruitment survey. *Marine and Coastal Management*, Department of Environmental Affairs and Tourism, WG/JUN99/PEL/19 (unpublished).

Baust, S., Teh, L., Harper, S. & Zellar, D. (2015). South Africa's marine fisheries catches (1950-2010). *Fisheries Catch Reconstruction for South Afica*, **23**(2), 129-150.

Bhardwaj, K., Verma, N., Trivedi, R.K., Bhardwaj, S. & Shukla, N. (2016). Significance of ratio of omega-3 and omega-6 in human health with special reference to flaxseed oil. *International Journal of Biological Chemistry*, **10**, 1-6.

Beckley, L.E. & Van der Lingen, C.D. (1999). Biology, fishery and management of sardines (*Sardinops sagax*) in southern African waters. *Marine and Freshwater Research*, **50**(8), 955-978.

Bernard, E. & Bolatito, A.Y. (2016). Comparative study on the nutritional composition of the pink shrimp (*Penaeus notialis*) and tiger shrimp (*Penaeus monodon*) from Lagos lagoon, Southwest nigeria. *Cogent Food & Agriculture*, **2**, 1-7.

Berzaghi, P. & Riovanto, R. (2009). Near infrared spectroscopy in animal science production: principles and applications. *Italian Journal of Animal Science*, **8**(3), 39-62.

Blanco, M., Coello, J., Iturriaga, H. Maspoch, S. & De La Pezuela, C. (1998). Near infrared spectroscopy in the pharmaceutical industry, *Analyst*, *London Society of Public Analysts Then Royal Society of Chemistry,* **123**, 135-150.

Bligh E.G & Dyer W.J. A rapid method of total lipid extraction and purification**.** *Canadian Journal of Biochemistry and Physiology 1959.* **37**(8), 911-917.

Breck, J.E. (2014). Body composition in fishes: body size matters. *Aquaculture*, **433**, 40-49.

Cascant, M.M., Breil, C., Fabiano-Tixier, Chemat, F., Garrigues, S. & De la Guardia, M. (2018). Determination of fatty acids and lipid classes in salmon oil by near infrared spectroscopy. *Food Chemistry*, **239**, 865-871.

Cen, H. & He, Y. (2007). Theory and application of near-infrared reflectance spectroscopy in determination of food quality. *Trends in Food Science and Technology,* **18**, 72-83.

Challadurai, V. & Jayas, D.S. (2014). Near-infrared and spectroscopy. In: *Imaging with Electromagnetic Spectrum: Applications in Food and Agriculture* (edited by A. Manickavasagan & H, Jayasuriya). Pp. 96-100, New York: Springer Heidelberg.

Chauke, E., Chkrowska, E., Thaela-Chimuka, M.J. Chimuka, L., Nsengimana, H. & Tutu, H. (2008). Fatty acids composition in South African freshwater fish as indicators of food quality. *Water South A*frica, **34**(1), 119-126.

Chen, Y., Sun, D., Cheng, J. & Gao, W. (2016). Recent Advances for Rapid Identification of Chemical Information of Muscle Foods by Hyperspectral Imaging Analysis. *Food Engineering Reviews,* **8**(3), 336-350.

Cheng, J., Sun, D., Zeng, X. & Liu, D. (2013). Recent advances in methods and techniques for freshness quality determination and evaluation of fish and fish fillets: A Review. *Food Science and Nutrition*, **55**(7), 1012-1226

Cheng, J. & Sun, D. (2015). Rapid and non-invasive detection of fish microbial spoilage by visible near-infrared hyperspectral imaging and multivariate analysis. *LWT-Food Science and Technology*, **62**(2), 1060-1068.

Cheng, J. Qu, J., Sun, D. & Zeng, X. (2014a). Visible/near-infrared hyperspectral imaging prediction of textural firmness of grass carp (*Ctenopharyngodon idella*) as affected by frozen storage. *Food Research international*, **56**, 190-198.

Cheng, J., Sun, D., Zeng, X. & Pu, H. (2014b). Non-destructive and rapid determination of TVB-N content for freshness evaluation of grass carp (*Ctenopharyngodon idella)* by hyperspectral imaging. *Innovative Food Science and Emerging Technologies*. **21**. 179-187.

Chitra Som, R.S. & Radhakrishnan, C.K. (2013). Seasonal variation in the fatty acid composition of *Sardinella longiceps* and *Sardinella fimbriata*: Implications for nutrition and pharmaceutical industry. *Indian Journal of Marine Sciences*, **42**(2), 206–210.

Çibuk, S., Atasoy, N. & Kaptanoglu, S. (2014). The study of total lipid rate and fatty acids of pearl mullet (*Chalcalburnus tarichii* P.1181) and nutritional importancein van, Turkey. *Journal of Food and Nutrition Research*, **2**(1), 1-8.

Coetzee, J.C., Van der Lingen, C.D., Hutchings, L. & Fairweather, T.P. (2008). Has the fishery contributed to a major shift in the distribution of South African sardine? *ICES Journal of Marine Science*, **65**, 1676-1688.

Conklin-Brittain, N.L., Dierenfeld, E.S., Wrangham, R.W., Norconk, M. & Silver, S.C. (1999). Chemical protein analysis: a comparison of Kjeldahl crude protein and total ninhydrin protein from wild tropical vegetation. *Journal of Chemical Ecology*, **25**(12), 2601-2622.

Cozzolino, D., Fassio, A. & Gimenez, A. (2001). The use of near-infrared reflectance spectroscopy (NIRS) to predict the composition of whole maize plants. Journal of Science of Food and Agriculture, **81**(1), 142-146.

Cozzolino, D., Kwiatkowski, M.J., Parker, M., Cynkar, W.U., Dambergs, R.G. & Gishen, M. (2004), Prediction of phenolic compounds in red wine fermentations by visible and near infrared spectroscopy. *Analytica Chimica Acta*, **513**(1), 73-80.

Cozzolino, D., Flood, L., Bellon, J., Gishen, M. & De Barios Lopes, M. (2006a). Combining near infrared spectroscopy and multivariate analysis as a tool to differentiate different strains of *Saccharomyces cerevisiae*: a metabolic study. *Yeast*, **23**, 1089-1096.

Cozzolino, D., Delucchi, I., Kholi, M. & Vázquez, D. (2006b). Use of near infrared reflectance to evaluate quality characteristics in whole-wheat grain. *Agricultura Técnica*, **66**(4), 370-375.

Cozzolino D. & Murray I. (2012). A review on the application of infrared technologies to determine and monitor composition and other quality characteristics in raw fish, fish products, and seafood. *Applied Spectroscopy Revews*, **47**(3), 207–218.

Culley, M. (1971). Geographical distribution of sardines zoological classification, etc. In: *The Pilchard biology & Exploitation* (edited by A.G. Kerkut). Pp. 10-14. New York: Pergamon Press Ltd.

Da Silva, T.E., Detmann, E., de Oliveira Franco, M., Nobre Palma, M.N. & Cipriano Rocha, G. (2016). Evaluation of digestion procedures in Kjeldahl method to quantify nitrogen in analysis applied to animal nutrition. *Acta Scientiarum. Animal Sciences*, **38**(1), 45-51.

Dai, Q., Cheng, J., Sun, D., Pu, H., Zeng, X & Xiong, Z. Potential of visible/near-infrared hyperspectral imaging for rapid detection of freshness in frozen prawns. *Journal of Food Engineering*, **149**, 97-104.

Davies, T. (1998). The history of near infrared spectroscopic analysis: Past, present and future "From sleeping technique to the morning star of spectroscopy". *Analusis*, **26**(4), 17-19.

De Goede, J. (2004). Spatial and temporal variability in lipid content and gonadosomatic index of sardine (*Sardinops sagax*) in Southern Benguela ecosystem. Master of Technology Thesis. Cape Technikon. South Africa.

De Longeril, M., Salen, P., Laporte, F. & De Leiris, J. (2001). Alpha-linolenic acid in the prevention and treatment of coronary heart diseases. *European Heart Journal Supplements*, **3**, 26-32.

Decker, E.A. (2005). Lipids. In: *Handbook of food analytical chemistry*. *Water, proteins, enzymes, lipids and carbohydrates*, Vol. 1 (edited by T.E. Acree, E.A. Decker, M.H. Penner, D.S. Reid, S.J. Schwartz, C.F. Shoemaker, D.M. Smith R.E. Wrolstad & P.S. Sporns). New York: John Wiley & Sons Inc. Pp.

Dérgadin, K., Guillemain, A. & Roggo, Y. (2017). Comprehensive study of a handheld Raman spectrometer for the analysis of counterfeits of solid-dosage form medicines. *Journal of Spectroscopy*, **2017**, 1-13.

Dufour, E.J. (2008). Sterols and membrane dynamics. *Journal of chemical Biology*, **1**(1-4), 63-77.

Esbensen, H.H. (2002). Principal component analysis (PCA). In: *Multivariate Data Analysis-in practice,* 5th Edition, Pp.27–34

Fawole, O.O., Yekeen, T.A., Adewoye, S.O., Ogundiran, M.A., Ajayi, O.E. & Nwaiya M.N. (2013). Nutritional qualities and trace metals concentration of six fish species from Oba reservoir, Ogbomoso, Nigeria. *African Journal of Food Science*, **7**(8), 246-252.

Flaete, P. O., Haartveit, E. Y., & Vadla, K. (2006). Near infrared spectroscopy with multivariate statistical modeling as a tool for differentiation of wood from tree specieswith similar appearance. *New Zealand Journal of Forest Science*, **36**(2-3), 382–392.

Fearn, T. (1999). Least squares. *NIR news*, **10**(7), 13.

Fenton, M. (1992). Chromatographic separation of cholesterol in foods. *Journal of Chromatography*, **624**, 369-388.

Folch, J., Lees, M., and Sloane Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, **226**, 497-509.

Fowler, J.L. & Boyd, A.J. (1998). Transport of anchovy and sardine eggs and larvae from the western Agulhas Bank to the west coast during the 1993/94 and 1994/95 spawning seasons. *South African Journal of Marine Science,* **19**, 181-195.

Fulop, A., Krar, M. & HancsÓk, J. (2008). Application of NIR spectroscopy by determination of quality properties of vegetable oils and their derivatives. *Hungarian Journal of Industrial Chemistry Veszprém*, **36**(1&2), 35-38.

Gámez-Meza, N., Higuera-Ciapara, I., Calderon de la Barca, A.M., Vázquez Moreno, L., Noriega-Rodrígueza, J. & Angulo-Guerrero, O. (1999). Seasonal variation in the fatty acid composition and quality of sardine from *Sardinops sagax caeruleus* of the Gulf of California. *Lipids*, **34**(6), 639-642.

Gordon, M.H. (2003). Fats. In: *Encyclopedia of Food Sciences and Nutrition*. 2nd Edition. Pp 2287-2292.

Gordon, A. Finegold, C. Crissman, C.C. & Pulis, A. (2013). Fish production, consumption and trade in Sub-Saharan Africa: A Review Analysis. *Prepared by World Fish. Fish to 2030: Sub-Saharan Africa Fish Trade in a Changing Climate*.

Granata, L.A., Flick, G.J. & Martin, R.E. (2012). Surimi and fish protein isolates. In: *The Seafood Industry: Species, Products, Processing and Safety*. 2nd Edition. p. 123. United Kingdom: John Wiley & Sons Ltd.

Grant, W. & Bowen, B. (1998). Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity*, **89**, 415-426.

Guelpa, A. (2015). Maize endosperm texture characterisation using the Rapid Visco Analyser (RVA), X-ray micro-computed tomography (µCT) and micro-near infrared (microNIR) spectroscopy. PhD Thesis. Stellenbosch University, South Africa.

Guo, X., Wilmshurst, J.F. & Li, Z. (2010). Comparison of laboratory and field remote sensing methods to measure forage quality. *International Journal of Environmental Research and Public Health*, 73513-3530.

Hampton, S.L. (2014). Multidisciplinary investigation into stock structure of small pelagic fishes in southern Africa. PhD Thesis. University of Cape Town, South Africa.

Hantoush, A.A., Al-Hamadany, Q.H. & Al-Hassoon, A.S. (2014). Nutritional value of important commercial fish from Iraqi waters, Mesopot. *Journal of Marine Science*, **29**(1), 13-22.

Haughey, S.A., Galvin-King, P., Melechaux, A. & Elliott, C. (2015). The use of handheld near-infrared reflectance spectroscopy (NIR) for the proximate analysis of poultry feed and to detect melamine adulteration of soya bean meal. *Analytical Methods*, **7**, 181-186.

Hernandez-Martinez, M., Gallardo-Velazquez, T., Osovio-Revilla, L., Almaraz-Abarca, N. & Castaneda-Perez, E. (2014). Application of MIR-FTIR spectroscopy and chemometrics to the rapid prediction of fish fillet quality. *Journal of Food*, **12**(4), 369-377.

Herschel, W.F. (1800). Experiments on the refrangibility of the invisible rays of the sun. *Philosophical Transactions of the Royal Society of London*, **90**, 284–292.

Huang, H., Yu, H., Xu, H. & Ying, Y., (2008). Near infrared spectroscopy for on/in-line monitoring of quality in foods and beverages: A review. *Journal of Food Engineering*, **87**, 303-313.

Huss, H.H. 1988. Fresh fish: quality and quality changes. p 132, FAO. Rome, Italy.

Huss, H.H. 1995. Quality and quality changes in fresh fish. p. 348, FAO. Rome, Italy.

Hutchings, L., Van der Lingen, C.D., Shannon, L.J., Crawford, R.J.M., Verheye, H.M., Bartholomae, C.H., van der Plas, A.K., Louw, D., Kreiner, A., Ostrowski, M., Fidel, Q., Barlow, R.G., Lamont, T., Coetzee, J., Shillington, F., Veitch, J., Currie, J.C. (2009). Marine fisheries monitoring programmes in South Africa. *South African Journal of Science*, **105**, 182-192.

Jabeen, F. & Chaudhry, A.S. (2011). Chemical compositions and fatty acid profiles of three freshwater fish species. *Food Chemistry*, **125**, 991–996.

Johnson, M & Bradford, C. (2014). Omega-3, omega-6 and omega-9 fatty acids: Implications on cardiovascular and other diseases. *Journal of Glycomics & Lipidomics*, **4**(4), 123.

Karlsdottir, M.G., Petty, H.T. & Kristinsson, H.G. (2014). Oxidation in aquatic foods and analysis methods. In: *Antioxidants and Functional Components in Aquatic Foods*. (edited by H.G. Kristinsson). 1st Edition. Pp. 308-310. Gainesville: John Wiley & Sons Publishers.

Karoui, R. & de Baerdermaeker, J. (2007). A review of the analytical methods coupled with chemometric tools for the determination of the quality and identity of dairy products. *Food Chemistry*, **102**, 621-640.

Kennedy, E.J., Robinson, S.M.C., Parsons, G.J. & Castell, J.D. (2007). Effect of lipid source and concentration on somatic growth of juvenile green sea urchins, *Strongylocentrotus droebachiensis*. *Journal of the World Aquaculture Society*, **38**(3), 335-352.

Khodabux, K., L'Omelette, M.S.S., Jhaumeer-Laullo, S., Ramasami, P. & Rondeau, P. (2007). Chemical and near-infrared determination of moisture, fat and protein in tuna fishes. *Food Chemistry*, **102**, 669-670.

Kimiya, T., Sivertsen, A.H. & Heia, K. (2013). Vis/NIR spectroscopy for non-destructive freshness assessment of Atlantic salmon (*Salmo salar*) fillets. *Journal of Food Engineering*, **116**, 758-764.

Koubaa, A., Abdelmouleh, A., Bouain, A. & Mihoubi, N.B. (2010). Experimental and statistical investigations of the global chemical composition of six trawling fish of the Gulf of Gabès (Mediterranean Sea). *Journal of the Marine Biological Association of the United Kingdom*, 1-7.

Kreiner, A., Van der Lingen, C.D. & Freon, P. (2001). A comparison of condition factor and gonadosomatic index of sardine*Sardinops sagax* stocks in the northern and southern Benguela upwelling ecosystems, 1984-1999. *South African Journal of Marine Science,* **23***,* 123-134.

Kucha, C.T., Liu, L. & Ngadi, M.O. (2018). Non-destructive techniques and multivariate analysis for assessment of fat quality in pork and pork products: A review. *Sensors*, **18**(377), 1-23.

Lagrue, C. & Poulin, R. (2015). Measuring fish body condition with or without parasites: does it matter? *Journal of Fish Biology*, **87**, 836-847.

Lambert, Y. & Dutil, J.D. (1997). Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of Atlantic cod (Gadus morhua)? *Canadian Journal of Fisheries and Aquatic Sciences*, **54**(1), 104-112.

Lambert, Y. & Dutil, J.D. (2000). Energetic consequences of reproduction in Atlantic cod (*Godus morhua*) in relation to spawning level of somatic energy reserves. Canadian *Journal of Fisheries and Aquatic Sciences*, **57**, 815-825.

Latifa, G.A., Chakraborty, S.C., Begum, M., Nahid, M.N. & Farid, F.B. (2014). Nutritional quality analysis of Bangladesh fish species, *M. Tengra* (Hamilton-Buchanan, 1822) preserved with different curing methods in laboratory condition. *American Journal of Food and Nutrition***,** **2**(6), 100-107.

Lau, E.V., Gan, S. & Ng, H.K. (2010). Extraction techniques for polycyclic aromatic hydrocarbons in soils. *International Journal of Analytical Chemistry*, **2010**, 1-9.

Lau, C.S. (2007). Formulation and physical, chemical and sensory analysis of a novel flaxseed-enriched milk-based beverage to deliver omega-3 fatty acids. PhD Thesis. Human nutrition, foods and exercise. Virginia Poly Technic Institute and State University. Blacsburg.

Li, S., Li, Z. & Gong, J. (2010). Multivariate statistical analysis of measures for assessing the quality of image fusion. *International Journal and Data Fusion*, **1** (1), 47-66.

Li Vigni, M., Durante, C. & Cocchi, M. (2013). Exploratory data analysis. In: *Chemometrics in Food Science*. Pp. 55-126. Oxford, England: Elsevier.

Liu, D., Zeng, X. & Sun, D. (2013). NIR spectroscopy and imaging techniques for evaluation of fish quality - A review. *Applied Spectroscopy Reviews*, **48**, 609-628.

Loftas, T. (1995). Dimensions of need: An Atlas of food and agriculture. Food and Agricultural Organisation of the United Nations (FAO). Rome, Italy.

Lloret, J., Shulman, G. & Love, R. M. (2014). Description of condition indicators. In: Condition and health indicators of exploited marine fishes. John Wiley & Sons Ltd. Chichester. USA.

Louw, G.G., Van der Lingen, C.C. & Gibbons, M.J. (1998). Differential feeding by sardine *Sardinops sagax* and anchovy *Engraulis capensis* recruits in miced shoals. *South African Journal of Marine Science*, **19**, 227-232.

Love, R. M., (1988). The Food Fishes. Their intrinsic variation and practical implications. Farrand Press, London, pp. 25-86.

Malegori, C., Marques, E.J.N, Tonetto de Freitas, S., Pimentel, M.F., Pasquini, C. & Casiraghi, E. (2017). Comparing the analytical performance of Mirco-NIR and FT-NIR spectrometer in the evaluation of acerola fruit quality, using PLS and SVM algorithms. *Talanta*, 165, 112-116.

Malseed, T. (2004). Stable isotope analysis of the food web supporting *sardinops sagax* in the waters off Esperance. Honours Thesis in Engineering environmnent. University of Western Australia, Australia.

Manley, M., McGoverin, C.M., Snyders, F., Muller, N., Botes, W.C. & Fox, G.P. (2013). Prediction of triticale grain quality properties based on both chemical and indirectly measured references methods using near-infrared spectroscopy. *Cereal Chemistry*. **90**(6), 540-545.

Manley, M. (2014). Near-infrared spectroscopy and hyperspectral imaging: non-destructive analysis of bilological materials, *Chemical Society Reviews*, **43**, 8200-8214.

Martens, H. & Naes, T. (1989). *Analysis of Quality, An introduction*. New York, USA: John Wiley & Sons Ltd.

Massart, D.L. (1998). Data handling in science and technology series, part A. In: *Handbook of Chemometrics and Qualimetrics* (edited by B. Vandeginste, L. Buydens, S. De Jong, P. Lewi & J. Smeyers-Verbeke). Pp. 519-556.

Mateos, H.T. (2012). The effects of feed supplement with omega-3 polyunsaturated fatty acids on cultured abalone. PhD in Biomedical Sciences Thesis. Victoria University, Australia.

Mika, A., Gołębiowski, M., Skorkowski, E.F. & Stepnowski, P. (2012). Composition of fatty acids and sterols composition in brown shrimp Crangon crangon and herring Clupea harengus membras from the Baltic Sea. *Oceanological and Hydrobiological Studies*, **41**, 57–64.

Miller, D.C.M., Moloney, C.L., Van der Lingen, C.D., Lett, C., Mullon, C. & Field, J.G. (2006). Modelling the effects of physical-biological interactions and spatial variability in spawning and nursery areas on transport and retention of sardine (*Sardinops sagax*) eggs and larvae in the southern Benguela ecosystem. *Journal of Marine Systems,* **61**, 212-229.

Min, D.B. & Ellefson, W.C. (2010). Fat analysis. In: *Food Analysis Laboratory Manual*, 2nd Edition (edited by S. Nielsen), Pp. 117-132. USA: Springer.

Mobley, P.R., Kowalski, B.R., Bro, R. (1996). Review of chemometrics applied to spectroscopy. *Review of Chemometrics Applied to Spectroscopy:* 1985-95 Part 1 , **31**, 73-124.

Mojekwu, T.O. & Anumudu, C.I. (2015). Advanced techniques for morphometric analysis in fish. *Journal of Aquaculture Research and Developmen*t, **6**(8), 1-6.

Moore, J.C., DeVries, J.W., Lipp, M., Griffiths, J.C. & Abernethy, D.R. (2010). Total protein methods and their potential utility to reduce the risk of food protein adulteration. *Comprehensive Reviews in Food Science and Food Safety*, **9**, 330–357.

Mraz, J. (2011). Lipid quality of common carp (*Cyprinus carpio*) in pond culture. Licentiate Thesis. Swedish University of Agricultural Sciences.

Müller, J. (2014). Dumas or Kjeldahl for reference analysis? *Comparison and considerations for nitrogen/protein analysis of food and feed*. P/N 1026738, Issue 1.

Muir, J.F. (2013). Fish, feeds and food security. *Animal Frontiers*. **3**(1), 28-34.

Murphy, B.R., Brown, M.L. & Springer, T.A. (1990). Evaluation of the relative weight (W_r) index with new applications to walleye. *North American Journal of Fisheries Management*, **10**(1), 85-97.

Mustać, B. & Sinovčić, G. (2009). Comparison of mesenteric and tissue fat in relation to sexual cycle of the sardine, *Sardina pilcardus* (Walb., 1972), in the eastern Middle Adriatic fishery grounds (Croatia). *Journal of Applied Ichthyology*, **25**, 595-599.

Naes, T. & Isaksson, T. (1991). SEP or RMSEP, which is best? *NIR news*, **2**, 16.

Nawrocka, A. & Lamorska, J. (2013). Determination of food quality by using spectroscopic methods. *Advances in Agrophysical Research*, 348-362.

Nisa, K. & Asadulla, K. (2010). Seasonal variation in chemical composition of the Indian mackerel (*Rastrelliger kanagurta*) from Karachi coast. *Iranian Journal of Fisheries Sciences*, **10** (1), 47-74.

Njuguna, J., Vanli, O.A. & Liang, R. (2015). A review of spectral methods for dispersion characterization of carbon nanotubes in aqueous suspensions. *Journal of Spectroscop*y, **2015**, 1-11.

Norris, K.H. (1989). Definition of NIRS Analysis. In: *Near Infrared Reflectance Spectroscopy (NIRS): Analysis of Forage Quality* (edited by G.C. Marten, J.S. Shenk & F.E. Bartondt II). Pp. 6, 12. Agriculture Handbook No 643. United States Department of Agriculture.

O'Brien, N.A., Hulse, C.A., Friedrich, D.M., Van Milligen, F.J., Von Gunten, M.K., Pfeifer, F. & Siesler, H.W. (2012). Miniature near infrared INIR) spectrometer engine for handheld applications. Next-Generation Spectroscopic Technologies V (edited by M.A. Druy, R.A. Crocombe), Proceedings of SPIE, Vol. 837404.

O'Brien, N.A., Hulse, C.A., Pfeifer, F. & Siesler, H.W. (2013). Near infrared spectroscopic authentication of seafood. *Journal of Near Infrared Spectroscopy*, **21**, 299-305.

Olivos-Trujillo, M., Gajardo, M.A., Salvo, S., Gonzalez, A.Z. & Muno, C. (2015). Assessing the stability of parameters estimation and prediction regression methods for estimating seed oil content in *Brassica napus L*. using NIR spectroscopy. *Santige De Chile*, **28**, 25-29.

Ono, R. (2016). Human history of maritime exploitation and adaptation process to coastal and marine environments- A view from the case of Wallacea and the Pacific. *Applied Studies of Coastal and Marine Environments*, 390-415.

Osborne, B.G., Fearn, T. & Hindle, P.H. (1993). Practical NIR Spectroscopy with applications in food and beverage analysis, 2nd edition, Pp. 1-220. England: Longman Group UK Limited.

Pasikatan, M.C., Steele, J.L., Spillman, C.K. & Haque, E. (2001). Near infrared reflectance spectroscopy for online particle size analysis of powders and ground materials. *International Journal of Near Infrared Spectroscopy*, **9**, 153-164.

Pasquini, C. (2003). Near infrared spectroscopy: Fundamentals; practical aspects and analytical applications. *Journal of Chemistry*, **14**(2). 198-219.

Pearson, K. (1901). On lines and planes of closest fit to systems of points in space. *The London, Edinburgh and Dublin Philosophical Magazine and Journal of Science*, **2**(11), 559-572.

Pere-Trepad, E., Olivella, L., Ginebreda, A., Caixach, J. & Jauler, R. (2006). Chemometrics modelling of organic contaminants in fish and sediment river samples. *Science of the total environment*, **371**, 223-237.

Perrson, J. (2008). Optimizing the Kjeldahl digestion. In: *Handbook for Kjeldahl Digestion: A recent review of the classical method with improvements from FOSS*, 4th ed. Pp. 15-33. Denmark: FOSS, DK-3400-Hillroed.

Prajapati, P., Solanki, R., Modi, V. & Basuri, T. (2016). A brief review on NIR spectroscopy and its pharmaceutical applications. *International Journal of Pharmaceutical Chemistry and Analysis*, **3**(3), 117-123.

Price, P.T., Nelson, C.M. & Clarke, S.D. (2000). Omega-3 polyunsaturated fatty acid regulation of gene expression. *Current Opinion in Lipidology*, **11**(1), 3-7.

Prieto, J., Pawluczyk, O., Dugan, M.E.R. & Aalhus, J.L. (2017). A review of the principles and applications of near-infrared spectroscopy to characterize meat products. *Applied Spectroscopy*, **71**(7), 1403-1426.

Quintanilla, L.F. and Pérez N. (2000). Spawning frequency of *Sardina pilchardus* (Walb.) off the Spanish North Atlantic coast in 1997. *Fisheries Research,* **45**(1), 73-79.

Raubenheimer, D., Simpson, S., Sanchez-Vazquez, J., Huntingford, F., Kandri, S. & Jobling, M. (2012). Nutrition and Diet Choice. In: *Aquaculture and behaviour* (edited by F. Huntingford, M. Jobling & S. Kandri). p. 151. United Kingdom: Blackwell Publishing Ltd.

Rayment, I. & Holden, H. M. (1994). The three dimensional structure of a molecular motor. *Trends in Biochemical Sciences*, **19**(3), 99-142.

Reed, C., Mackenzie, K. & van der Lingen, C.D. (2012). Parasites of South African sardines, *Sardinops sagax* and an assessment of their potential as biological tags. *Fish Pathology,* **32** (2), 41. South Africa.

Rogalski, A. (2002). Infrared detectors: an overview. *Infrared Physics and Technology*, **43**, 187-210.

Rustan, A.C. & Devron, C.A. (2005). Fatty Acids: structures and properties. In: *Encyclopedia of Life Science.* Pp. 1-2, Norway: John Wiley & Sons, Ltd.

Sader, A.P,O., Oliveira, S.G. & Berchielli, T.T. (2004). Application of Kjeldahl and Dumas combustion methods for nitrogen analysis. *Archives of Veterinary Science*, **9**, 73–79.

Saeed, M., Ali Reza, A., Hamid, F., Maryan, S. & Nuria, P. (2012). Determination of protein and moisture in fishmeal by near-Infrared reflectance spectroscopy and multivariate regression based on partial least squares. *Iran Journal of Chemical Engineering,* **31**(3). 51-59.

Sampels, S., Strandvik, B. & Pickova, J. (2009). Processed animal products with emphasis on polyunsaturated fatty acid content. *European Journal of Lipid Sciences and Technology* in press, 1-20.

Santos, C.A.T., Lopo, M., Pascoa, N.M.J., Lopes, J.A. (2013). A review on the apllications of portable near infrared spectrometers in the agro-food industry. *Applied Spectroscopy*, **67**(11), 1215-1218.

Savitri, S. (2011). Heat denaturation of proteins from Atlantic salmon (*Salmor salar*). Master's Thesis. University of Stavanger, Norway.

Sekar, S., Shafie, S.R., Prasadam, I., Crawford, R., Panchal, S.K., Brown, L. & Xiao,Y. (2017). Saturated fatty acids induce development of both metabolic syndrome and osteoarthritis in rats. *Scientific Reports*, **7**, 1-11.

SK Shahina, B, Hareesh, K. & Srinivasulu, M.R. (2016). Evaluation of nutritional staus of Penaeid prawns through proximate composition studies. *International Journal of Fisheries and Aquatic Studies*, **4**(1), 13-19.

Shearer, K.D. (1994). Factors affecting the proximate composition of culture fishes with emphasis on salmonids. *Aquaculture*, **119**(1), 63-88.

Shulman, G.E.; Love, R.M. (1999). Indicators of fish condition. In: *Advances in Marine Biology: The Biochemical Ecology of Marine Fishes*, Volume 36 (edited by A.J. Southward, P.A. Tyler & C.M. Young), Pp. 205-206. Academic Press Limited, California: USA.

Šimat, V., Bogdanović, T. Krželj M. (2012). Application of principal component analysis to characterize the effect of catching ground on post-mortem quality changes in ice stored sea bream (*Sparus aurata*, L) and Bogue (*Boops boops*, L.). *Croation Journal of Food Technology, Biotechnology and Nutrition*, **7**(Special issue), 14-17.

Simopoulos, A.P. (2000). Human requirement for n-3 polyunsaturated fatty acids. *Poultry Science*, **79**(7), 961-970.

Sreelekshmi, S.; Benno Pereira, F.G. & Binil, V.S. (2017). Morphometric evaluation of an indigenous fish belonging to genus Devario from three river systems of South Kerala. *Journal of Biodiversity and Environmental Sciences*, **10**(2), 106-114.

Strobel, C., Jahreis, G. & Kuhnt, K. (2012). Survey of n-3 and n-6 polyunsaturated fatty acids in fish and fish products. *Lipids in Health & Disease*, **11**.

Sumi, E.S., Vijayan, D.K., Jayarani, R., Navaneethan, R. & Mathew, S. (2016). Biochemical composition of Indian common small pelagic fishes indicates richness in nutrients capable of ameliorating malnutrition and age-associated disorders. Journal of Chemical Biology & Therapeutics, **1**(2), 1-5.

Sutharshiny, S. & Sivashanthini, K. (2011). Proximate Composition of three species of scomberoides fish from Sri Lankan waters. *Asian Journal of Clinical Nutrition*, 1–9.

Tang, H., Chen, L., Xiao, C. & Wu, T. (2009). Fatty acid profiles of muscle from large yellow croaker (*Pseudosciaena crocea* R.) of different age. *Journal of Zhejiang University Science B*, **10**(2), 154-158.

Tarley, C.R.T., Visentainer, J.V., Matsushita, M. & de Souza, N.E. (2004). Proximate composition, cholesterol and fatty acid profile of canned sardines (*Sardinella brasiliensis*) in soybean oil and tomato sauce. *Food Chemistry,* **88**,1-6.

Toivo, J., Phillips, K., Lampi, A.M. & Piironen, V. (2001). Determination of sterols in foods: Recovery of free, esterified and glycosidic sterols. *Journal of Food Composition and Analysis*, **14**, 631-643.

Totsch, S.K., Waite, M.E. & Sorge, R.E. (2015). Molecular and cell biology of pain. Progress in Molecular and Cell Biology Translational Science; **131**, 435-469.

Trattner, S. (2009). Quality of lipids in fish fed vegetable oils. Effect of bioactive compounds on fatty acid metabolism. PhD Thesis. Swedish university of Agricultural Sciences. Sweden.

Tsegay, T., Natarajan, P. & Zeleam, T. (2016). Proximate and mineral composition of some commercially important fish species of tekeze reservoir and lake Hashege, Ethiopia, Int. *Journal of Fisheries and Aquatic Studies*, **4**(1), 160-164.

Twatwa, N.M., Van der Lingen, C.D., Drapeau, L., Moloney, C.L. & Field, J.G. (2005). Characterising and comparing the spawning habitats of anchovy *Engraulis encrasicolus* and sardine *Sardinops sagax* in the southern Benguela upwelling ecosystem. *African Journal of Marine Science,* **27**(2), 487–499.

Uddin, M., Ishizaki, S., Okazaki, E. & Tanaka, M. (2002). Near-Infrared reflectance spectroscopy for determining end-point temperature of heated fish and shell fish meats. *Journal of Science of Food and Agriculture*, **82**, 286-292.

Van der Lingen, C.D. (1994). Effect of particle size and concentration on the feeding behavior of adult pilchard, *Sardinops sagax*. *Marine Ecology Progress Series*, **109**, 1-13.

Van der Lingen, C.D. (1998). Gastric evacuation feeding, periodicity and daily ration of sardine *Sardinops sagax* in the southern Benguela upwelling ecosystem. *South African Journal of Marine Science*, **19**(1), 305-316.

Van der Lingen, C.D. (1999). The feeding ecology of, and carbon and nitrogen budgets for, sardine *Sardinops sagax* in the Southern Benguela upwelling ecosystem. PhD Thesis. University of Cape Town, South Africa.

Van der Lingen, C.D. (2002). Diet of sardine *Sardinops sagax* in the southern Benguela upwelling ecosystem. *South African Journal of Marine Science*, **24**(1), 301-316.

Van der Lingen, C.D. & Huggett, J.A. (2003). The role of ichthyoplankton surveys in recruitment research and management of South African anchovy and sardine. The Big Fish Bang, The Proceedings of the 26th Annual Larval Fish Conference (edited by H.I. Howard & A.B. Skiftesvik), Institute of Marine Research, 303-343.

Van der Lingen, C.D. & Hutchings, L. (2005). Estimating the lipid content of pelagic fish in the southern Benguela by visual assessment of their mesenteric fat. *African Journal of Marine Science*, **27** (1), 45-53.

Van der Lingen, C.D., Hutchings, L. & Field, J.G. (2006). Comparative trophodynamics of anchovy *Engraulis encrasicols* and sardine *Sardinops sagax* in the southern Benguela: are species alternations between small pelagic trophodynamically mediated? *African Journal of Marine Science*, **28**(3&4),465-477.

Vélez-Arellano, N., F. García-Domínguez, D. B. Lluch-Cota, J. L. GutiérrezGonzález & R. Sánchez-Cárdenas (2015). Histological validation of morphochromatically-defined gonadal maturation stages of green abalone (*Haliotis fulgens*) Philippi, 1845 and pink abalone (*Haliotis corrugata*) Wood, 1828. *International Journal of Morphology*, **33**(3), 1054-1059.

Venugopal, V. & Shahidi, F. (2009). Structure and composition of fish muscle. *Food Reviews International*, **12**(2), 175-197.

Vongsvivut, J., Miller, M.R., McNaughton, D., Heraud, P. & Barrow, C.J. (2014). Rapid discrimination and determination of polyunsaturated fatty acid composition in marine oils by FTIR spectroscopy and multivariate data analysis. *Food and Bioprocess Technology: An International Journal*, **7**(8), 2410-2422.

Wang, L. & Mizaikoffi, B. (2008). Application of multivariate data-analysis techniques to biomedical diagnostics based on mid-infrared spectroscopy. *Analytical and Bioanalytical Chemistry*, **391**(5), 1642-1664.

Wang, H., Zhang, F., Cao, J., Zhang, Q. & Chen, Z. (2013). Proteolysis characteristics of sarcoplasmic, myofibrillar and stromal proteins from grass carp and antioxidant of their hydrolysates. *Food Science and Biotechnology*, **22**(2), 531-540.

Weatherby, K. & Carter, D. (2013). Chomera veila. *Advances in Applied Microbiology*, **85**, 119-144.

Weeranantanaphan, J., Downey, G., Allen, P. & Sun, D. (2011). A review of near infrared spectroscopy in muscle food analysis: 2005-2010. *Journal of Near Infrared Spectroscopy*, **19**, 61-104.

Wehling, R.L. (1994). Infrared spectrometry. In: Introduction to the chemical analysis of foods (edited by S.S. Nielsen). Pp. 344, 346-347, 349. Boston, MA: Jones and Bartlett Publishers.

Wehrens, R. (2011). Multivariate regression. In: *Chemometrics with R, Multivariate Data Analysis in the Natural Sciences and Life Sciences* (edited by R. Gentleman, K. Hornik & G. Parmigiani). P. 153; Berlin, Heidelberg: Springer-Verlag.

Westad, F., Bevilacqua, M. & Marini, F. (2013). *Regression*. In: *Chemometrics in Food Science*. Pp. 127-170. Oxford, UK: Elsevier.

Wetzel, D.L.B. (1998). Analytical near infrared spectroscopy. In: Instrumental methods in food and beverage analysis (edited by D.L.B. Wetzel & G. Charalambous). Pp. 159, 166-170, 188. New York: Elsevier.

Whittle, K. J. (2009). Marine organisms as food, forage, industrial and medical products. In: Encyclopedia of Life Support Systems (edited by P. Safran), Volume 1. Pp. 144-150.

Wiesner, K., Fuchs, K., Gigler, A.M. & Pastusiak, R. (2014). Trends in near infrared spectroscopy and multivariate data analysis from an industrial perspective. *Procedia Engineering*, **87**, 867-870.

Williams, P.C. (1991). Prediction of wheat kernel texture in whole grains by near-infrared transmittance. *Cereal Chemistry*, **68**, 112-114.

Williams, P., Geladi, P., Fox, G. & Manley, M. (2009). Maize kernel hardness classification by near infrared (NIR) hyperspectral imaging and multivariate analysis. *Analytica Chimica Acta,* **653**, 121-130.

Wold, S., Esbensen, K., Geladi, P. (1987). Principal component analysis. *Chemometrics Intelligent Laboratory Systems*, **2**(1-3), 37-52.

Wold, S., Sjostrom, M. & Eriksson, L. (2001). PLS-regression: a basic tool of chemometrics. *Chemometrics Intelligent Laboratory Systems*, **58**, 109-130.

Xiccato, G., Trocino, A., Tulli, F. & Tibaldi, E. (2004). Prediction of chemical compositon and origin identification of European sea bass (*Dicentrarchus labrax* L) by near infrared reflectance spectroscopy (NIRS). *Food Chemistry*, **86**, 275-281.

Yang, H. Griffiths, P.R., Tate, J.D. (2003). Comparison of partial least squares regression and multi-layer neural networks for quantification of non-linear systems and applications to gas phase fourier transform infrared spectra. *Analytica Chemica Acta*, **489**, 125-136.

Zaldivar, J. (1998). Comparison between Kjelfoss and Leco techniques for measuring protein content of fish meals. International Fish meal & Oil Manufacturers Association. Resarch Number: 1998-4, CORPESCA, Chile.

Zlatanos, S. & Lastaridis, K. (2007). Seasonal variation in the fatty acid composition of three Mediterranean fish – sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicholus*) and picarel (*Spicara smaris*). *Food Chemistry*, **103**, 725-728.

Zorica, B. & Keč, V.Č. (2012). Age, growth and mortality of the garfish, *Belone belone* (L 1761) in the Adriatic Sea. *Journal of the Marine Biological Association of the United Kingdom*, **93**(2), 365-372.

Zumba, J. & Rodgers, J. (2016). Cotton micronaire measurements using small portable near-infrared (NIR) analyzers. Applied *Spectroscopy*, **70**(5), 794-803.

3 CHAPTER THREE

APPLICATION OF NEAR INFRARED (NIR) SPECTROSCOPY TO THE EXPLORATION OF SEX AND MORPHOPHYSIOLOGICAL INDICATORS ON WHOLE AS WELL AS HOMOGENISED SOUTH AFRICAN SARDINES (*Sardinops sagax ocellatus*)

Abstract

The feasibility of near-infrared (NIR) spectroscopy as a non-destructive method to classify sardine (*Sardinops sagax ocellatus*) samples according to sex and morphophysiological (gonad stage and mesenteric fat) porperties was evaluated in this study. Sex as well as morphophysiological properties of SA sardine samples were used as indicators when applying near-infrared (NIR) spectroscopy and principal component analysis (PCA). Whole (n = 116), and homogenised (n = 97) sardines respectively, were scanned using a hand-held NIR spectrophotometer with a wavelength range of 908 to 1680 nm. The morphophysiological properties and sex were determined using macroscopic visual assessment method as developed by DAFF. By combining the NIR data and morphophysiological information, PCA was generated to remove outliers and was effective in identifying and grouping sardines according to different sex, fat stage and gonad stage for the whole sample set scanned on the skin. When looking at the homogenised sample set, PCA provided a rough discrimination only for the fat stages, with gender and gonad stage not showing any distinct clustering. These results demonstrated that NIR spectra, PCA, combined with mesenteric fat groupings could be used as a reliable procedure to group sardine samples according to fat stages, regardless of being scanned as a whole fish on the skin or homogenised.

3.1 Introduction

The South African (SA) sardine (*Sardinops sagax ocellatus*) also popularly known as pilchard (Grant *et al*., 1998; Bianchi *et al*., 1999); is an economically valuable forage species in the Benguela ecosystem (van der Lingen, 2002; Coetzee *et al*., 2008; Reed *et al*., 2012). There are historically five species in which sardine co-exist in the sardinops genus; *S. ocellatus* off Southern Africa, *S. neopilchardus* off southern Australia and New Zealand, *S. sagax* off the west coast of South America, *S. caerulleus* off the west coast of North America and lastly, *S. melanostictus* off Japan (Whitehead, 1985).

SA sardines are distributed in the Northern and Southern Benguela Ecosystem from southern Angola to KwaZulu-Natal on the north-east coast Beckley & Van der Lingen, 1999). Their range extends from southern Namibia, where an intense perennial upwelling cell off Lüderitz (27 ˚S) exists and forms a thermal barrier to exchange with the Namibian sardine population (Schulein *et al*., 1995; Lett *et al*., 2007) to Richard's Bay on South Africa's northeast coast (Beckley & Van der Lingen, 1999). The main nursery area for sardine off South Africa is between the Orange River and St Helena Bay on the West Coast (Armstrong & Thomas, 1989).

The commercial sardine fishery commenced in 1943 during World War II, following a demand for canned fish in SA (Agenbag *et al*., 2003; Coetzee *et al*., 2008; De Moor & Butterworth, 2015). Sardine landings have shown fluctuations in abundance and catch landings (Van der Lingen & Durholtz, 2003; De Moor & Butterworth, 2015). Increases in annual catches continued, peaking at around 400,000 t in 1962 and subsequently declining rapidly as a result of over exploitation (Coetzee *et al*., 2008; van der Lingen, 2002). Landings substantially increased in the 21st century to 250 000 t in 2002–2004 as surveys indicated good sardine recruitment and subsequent rapid growth in the size of the population (van der Lingen & Durholtz, 2003; de Moor and Butterworth 2009). Several years of low sardine recruitment since 2004, resulted in a rapid depletion in the size of the sardine stock with sardine catches dropping to 90 000 t between 2008 and 2014, to only 45 560 in 2017 and 65 000 t in 2018 (Fig. 3.1) (Coetzee *et al*., 2008a; Anon., 2016; Coetzee *et al*., 2018).

Due to economic importance of the SA sardine resource coupled with the variations in the sardine biomass (Barange *et al*., 1999), knowledge of stock structure is crucial to the management of fishery resources, as it is known to form the basic unit to implement appropriate management measures to ensure sustainability (Campbell, 2008). Identification of stock is critical in providing a biological understanding of stocks to fisheries management (Coyle, 1998; Begg *et al*., 1999; Begg & Waldman, 1999). The assessment of fish condition has become a standard practice in the management of fisheries, both at individual and population level. This practice has been used widely to both marine and terrestrial animals (Hussey *et al*., 2009).

One of the most critical considerations for sustainable fishery management is the estimation of the population reproductive status (Galindez *et al*., 2014). Understanding the reproductive status of sardine stock is crucial as it impacts recruitment (Knapp *et al*., 2014). Sex studies form an essential part of fishery biological investigations and hence the sustainability of the stock and its capacity for supporting commercial fishing (George, 1959; Knapp *et al*., 2014). It is therefore crucial for fisheries to measure body condition of fish to understand population dynamics (Brosset *et al*., 2015) and monitor the status of fish stock (Lambert & Dutil, 1997). The

assessment of fish condition has become a standard practice in management of fisheries, both at individual and population level (Hussey *et al*., 2009).

Each fish should be identified as reproductive or non-reproductive by macroscopic evaluation of ovaries and testes at different maturation stages (Fontoura *et al*., 2009). The identification of maturity and sex in fish species is laborious and requires a large sample group for dissection and macroscopic examination of their gonads (Murua *et al*., 2003; Murua, 2003). Physiological indicators measure energy reserves of fish more accurately than morphometric indices (Shulman & Love, 1999). Physiological (gonadosomatic) condition index is the macroscopic evaluation of maturity to determine the reproductive status of fish (McPherson *et al*., 2011; Brosset *et al*., 2015).

Visual assessment of mesenteric fat is a newly developed technique to examine fish condition (De Goede, 2004). Mesenteric fat measurement is the overall visual measure of the magnitude of fat deposited in the mesentery and is routinely applied to several "fatty" fish (McPherson *et al*., 2011). Sardine with high lipid content is considered to be in a better condition than sardine with low lipid content (De Goede, 2004). Fat amounts of sardines vary throughout the year (Mustać & Sinovčić, 2009; De Goede, 2004) and it is, therefore, necessary for the fish canning industry to have data on the monthly lipid content of sardine tissues readily collected (Mustać & Sinovčić, 2009). In a survey conducted by Larsen *et al*., (1997) between herring populations, it was established that large lipid variations existed within catches, inducing problems in commercial production. This was restricted by evaluating the lipid content of incoming raw material by visual characterisation of whole or gutted fish during landing. Incorrect assignment of maturity stage can lead to problems in fisheries management since the managers need to know the real number of spawning fish in a population to be able to manage fishing effort effectively (Costa, 2000).

Data from the morphophysiological analysis is commonly analysed using multivariate statistics such as principal component analysis (PCA) (Kocosky *et al*., 2009). PCA is superior to univariate analysis because it simultaneously considers all relationships among all dimensions rather than extracting morphologics individually (Bookstein *et al*., 1985). PCA is an effective tool for exploratory data analysis that is applied to NIR data (amongst others) to reduce the dimensionality of large data sets (Williams *et al*., 2012; Jolliffe & Cadima, 2016; Pořízka *et al*., 2018). The data is reduced to a much smaller number of principal components (PCs) which can be used as an exploratory technique. In PCA, the first PC conveys the most significant amount of information regarding the spectral variation, followed by the second PC and so forth. The use of

multivariate techniques such as PCA to assess morphometric indicators is gaining increased attention in stock identification (Mojekwu & Anumudu, 2015).

Attributes that group together can guide subsequent analytical designs, i.e. if observations cluster by gender as an example. Considering the commercial, social and economic importance of sardine in the fishery sector, it is necessary to assess all changes in sardine population so that it would be possible to respond in time to all possible adverse changes and to recommend preventive measures for protecting this specie (Mustać & Sinovčić, 2010).

Previous studies in which NIR spectroscopy was applied to investigate similar indicators are the classification of Chinook salmon (*Oncorhynchus tshawytsscha*) as female or male, as well as being mature versus immature (Hampton *et al*., 2002) and determination of ovarian maturity in farmed white Sturgeon (*Acipenser transmontanus*) (Lu *et al*., 2010), to name a few. Corti *et al*. (1988) used multivariate techniques to investigate the distinctness and interrelationships of six stocks of common carp (*Cyprinus carpio*). PCA was able to group the components according to size and detect variation between strains in carp culter. In the current study, morphophysiological indicators were used along with near-infrared (NIR) spectrophotometric data, to explore South Africa sardine characteristics.

Although some indices are non-destructive, others necessitate the sacrifice of the fish sample i.e. mesenteric fat and gonad stage. This study aimed to use PCA applied on NIR spectra to indicate which morphophysiological indicators could be used to reveal the internal structure in a way that best describes the variance of a sample set consisting of whole fish scanned on the skin and homogenised sample.

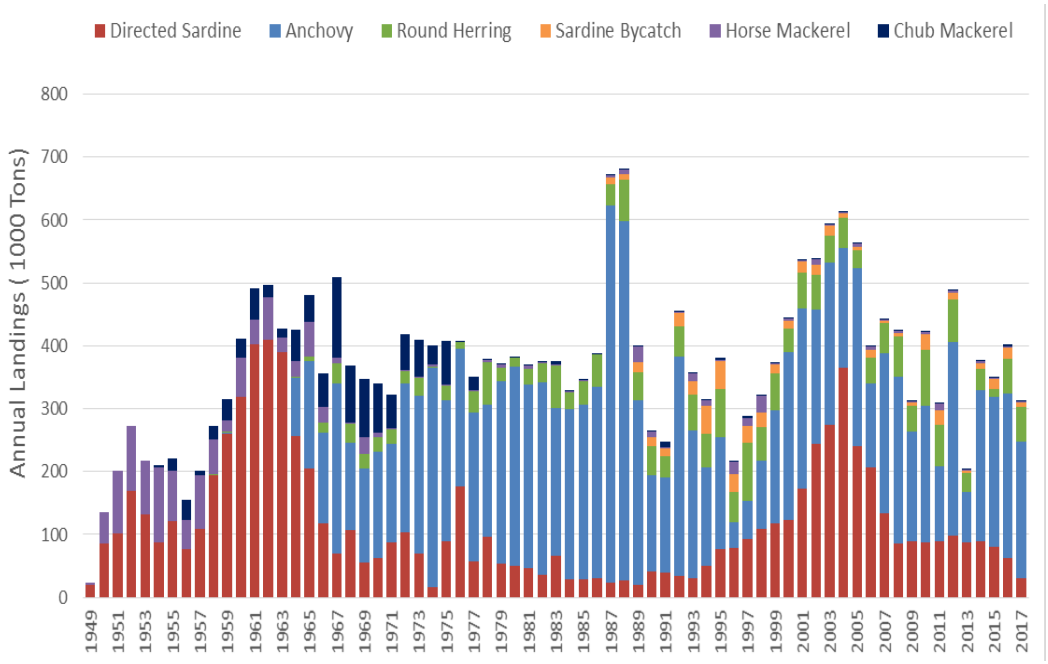


Figure 3.1: Annual landings of sardine and other small pelagic fish by the South African purse-seine fishery since 1949 (Coetzee *et al*., 2018).

3.2 Materials and methods

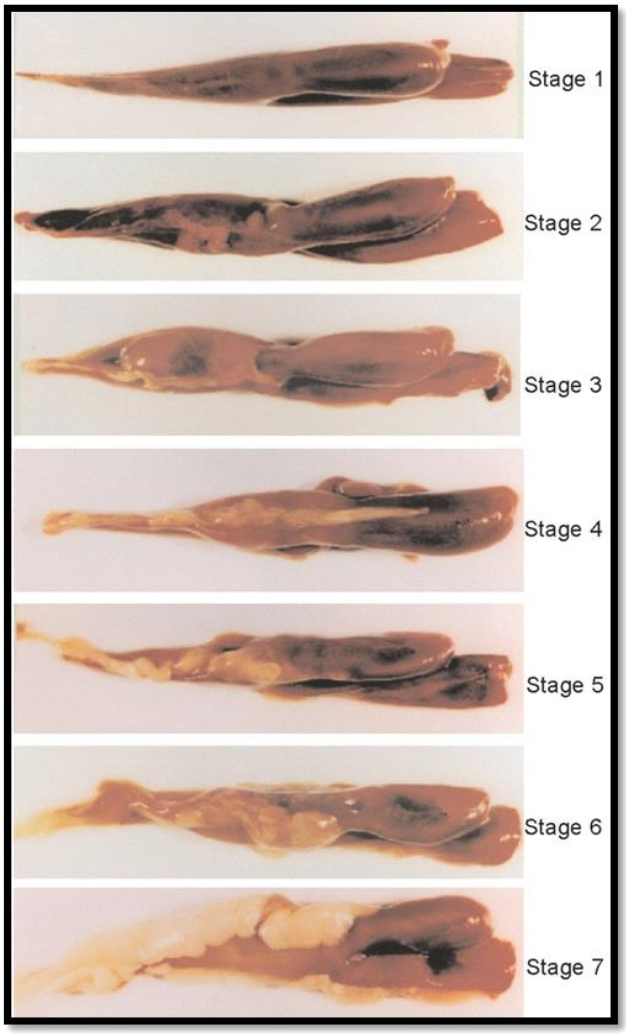
3.2.1 Samples

Sardine samples were procured along the west coast of South Africa from commercial purse seiners (fishing vessels) and DAFF research surveys. The fish samples were obtained from the Department of Agriculture, Forestry and Fisheries (DAFF; known as *Department of Agriculture*, Land Reform and Rural Development (DALRRD) from 2020), Cape Town, South Africa. There were two fishing dates: i.e. 28 June 2016 and 3 July 2017 with separate locations (Station A32578 Grid 4705A on 28 June and Station A32598 Grid 55-01 on 3 July). Once caught, the fish were covered in blue polyethylene bags and immediately frozen on board of the respective vessels. The fish samples were kept frozen and transported to Cape Peninsula University of Technology (CPUT), Department of Food Science and Technology laboratories where the samples were then stored in a freezer between -17 and -20°C until subjected to NIR spectral acquisition, morphophysiolgocial and sex examination.

3.2.2 Sex and Morphophysiological characteristics

- Sardine samples used in this study were given a unique number after they were thawed at ambient temperature. Each sample was cut open from the anus to the gills and the stomach, and other internal organs were removed. The following data were recorded for each sample:
- 1. Caudal length (CL) was measured in centimetres (cm) from the tip of the snout to the tip of the caudal vertebra (Fig. 3.1);
- 2. fish weight, i.e. the total body mass (g) of each sardine sample was weighed to the nearest 0.1 g using an electronic scale (PCB 3500-2 Kern, Germany), accurate to two decimals;
- 3. Sex of each sardine sample was determined using a macroscopic examination of the gonads (ovaries were examined according to colour, structure and clarity of the oocytes while testes were examined only according to colour and structure); 4. mesenteric fat (fat stage) of each sardine sample was assigned according to the criteria developed by van der Lingen and Hutchings (2005) to visually estimate the respective fat stages (Fig. 3.2); and
- 5. gonads of each sardine sample were removed from each sardine sample, and the respective reproductive maturity was determined using a macroscopic staging system, as described by van der Lingen and Hutchings (2005).

Figure 3 2: Caudal length measurement of *Sardinops sagax ocellatus*, using a measurement bolt, taken from the tip of the snout to the end of the caudal vertebra. Photo was taken using Huawei P30 Lite



Stage 1: Thin or invisible fat lines.

Stage 2: Depth greater than the width of one or more fat lines.

Stage 3: Pyloric fat noticeably thicker than the other fat lines.

Stage 4: Depth greater than width for all fat lines but no fat lobes present.

Stage 5: All fat lines slightly lobed but no overlap between lobes.

Stage 6: Obvious fat line lobes that show some overlap.

Stage 7: Large fat line lobes with lots of overlap and fundulus well covered with fat.

Figure 3 3: Description of fat stages for *Sardinops sagax ocellatus* adopted from (Van der Lingen & Hutchings, 2005).

3.2.3 Homogenising the fish samples

As the head and gut of each fish were removed at this stage, individual fish fillets were homogenised using a FOSS (Hillerød, Denmark, Knifetec 1095^{TM} DK-3400) sample mill at 20 000 rpm per 10 s. The homogenised samples were individually vacuum packed in plastic bags and stored at -17 to -20°C until analysed for morphological indices. For this chapter, only sex morphophysiological indicators were used for PCA in combination with NIR spectral analysis.

3.2.4 Near-infrared spectral data acquisition

NIR spectra of whole and homogenised sardine samples were acquired with the use of an ultra-compact hand-held Viavi's MicroNIR 1700 spectrophotometer. The external white reference was used as the diffuse reflectance standard (Viavi Solutions Inc., Milpitas, CA, USA). Scanning took place in reflectance mode at 6.2 nm intervals within the wavelength range of 908 to 1650 nm. Each scan was the average of 64 individual scans (scanning parameter). MicroNIR software (Viavi Solutions Inc., Milpitas, CA, USA) was used to perform the spectroscopic measurements.

Thawed, intact sardines were scanned on the skin, and the sample set consisted of 116 samples (n = 116). The scanning was conducted in triplicate (the anterior ventral, mid ventral and dorsal section) per sample and was later averaged. Additional spectra of a sample set (n = 97) consisting of the homogenised samples were acquired. These samples were placed individually in a custom-made hollowed-out Teflon (PFTE) disk [(Maizey's (Pty) Ltd, Kuilsriver, South Africa)] (Fig. 3.3b) that completely covered the contactable collar of the MicroNIR spectrophotometer upon scanning. The scanning was conducted in duplicate per sample and later averaged. The Teflon disc was wiped clean between consecutive samples.

All spectral data were saved in Microsoft Excel format for further analysis in The Unscrambler software (Camo ASA, Oslo, Norway) version 10.4.

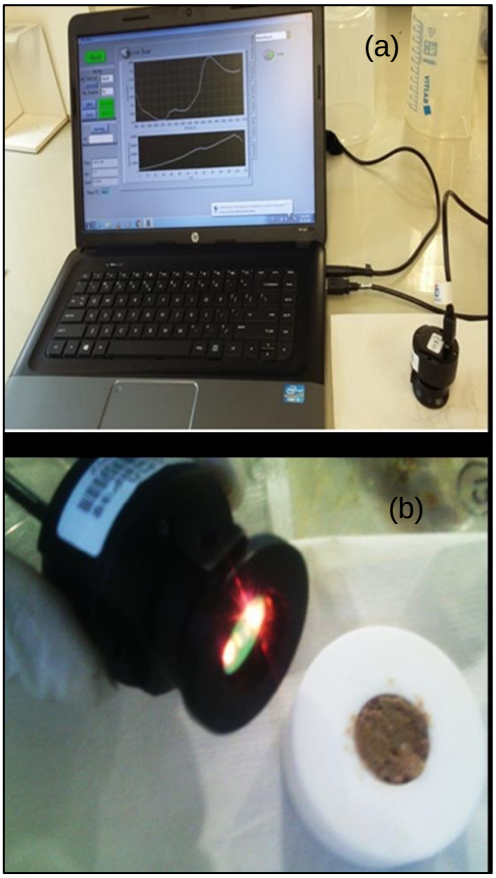


Figure 3.4: (a) Photo illustration the MicroNIR instrument connected to a laptop and (b) a MicroNIR next to a hollowed-out Teflon disk with a homogenised fish sample inside as it was used during the scanning of individual homogenised fish samples. Photos were taken using a Huawei P30 Lite.

3.2.5 Multivariate data analysis: exploratory stage using PCA

The mean centred and multiplicative scatter corrected (MSC) NIR data on both the whole sardine sample, as well as the homogenised sample set were subjected to PCA analyses. Individual scores were used to construct scatter plots to reveal groupings of the fish. PCA analyses were carried out using Unscrambler v.10.4 (Camo, Oslo, Norway) software.

3.3 Results and discussion

3.3.1 Whole sardines

3.3.1.1 NIR spectra

Figure 3.5a shows the absorbance spectra of the raw NIR spectral measurements of the entire sample set (n = 116) of whole fish scanned on the skin. The respective spectral data of whole fish scanned on the skin were pre-treated with a pre-treatment technique, i.e. multiplicative scatter correction (MSC) to eliminate the effects of physical phenomena such as the light scattering, commonly observed when samples consist of particles with different sizes and shapes (Isaksson & Naes, 1988, Helland *et al*., 1995; Manley, 2014). Application of pre-processing methods such as MSC improves data analysis (exploratory analysis), adjusts baseline shifts resulting in better linear fit, better spectral interpretability and improved prediction results compared to uncorrected data (Rinnan *et al*., 2009; Almendigen *et al*., 2000). Figure 3.5b shows how the light scattered corrected samples differ from the original spectra Fig. 3.5a as the bands are now far better defined.

Sardine catches are tested at field stations next to the factories where most of the catch is landed for processing, hence the scanning on the skin for biological characteristics (Fairweather *et al*., 2006). A practical, rapid and non-invasive technique to analyse sex and morphophysiological characteristics of fish has considerable potential to assist with the management of fish stocks and improving quality thereof (Simon *et al*., 2016). Prediction of condition indices by non-invasive analysis of whole fish is more challenging than processed tissues because fish skin absorbs and reflects electromagnetic radiation in the NIR region, as a consequence makes it difficult to obtain representative measurements from the interior of the fish muscle (Lin *et al*., 2006; Simon *et al*., 2016)

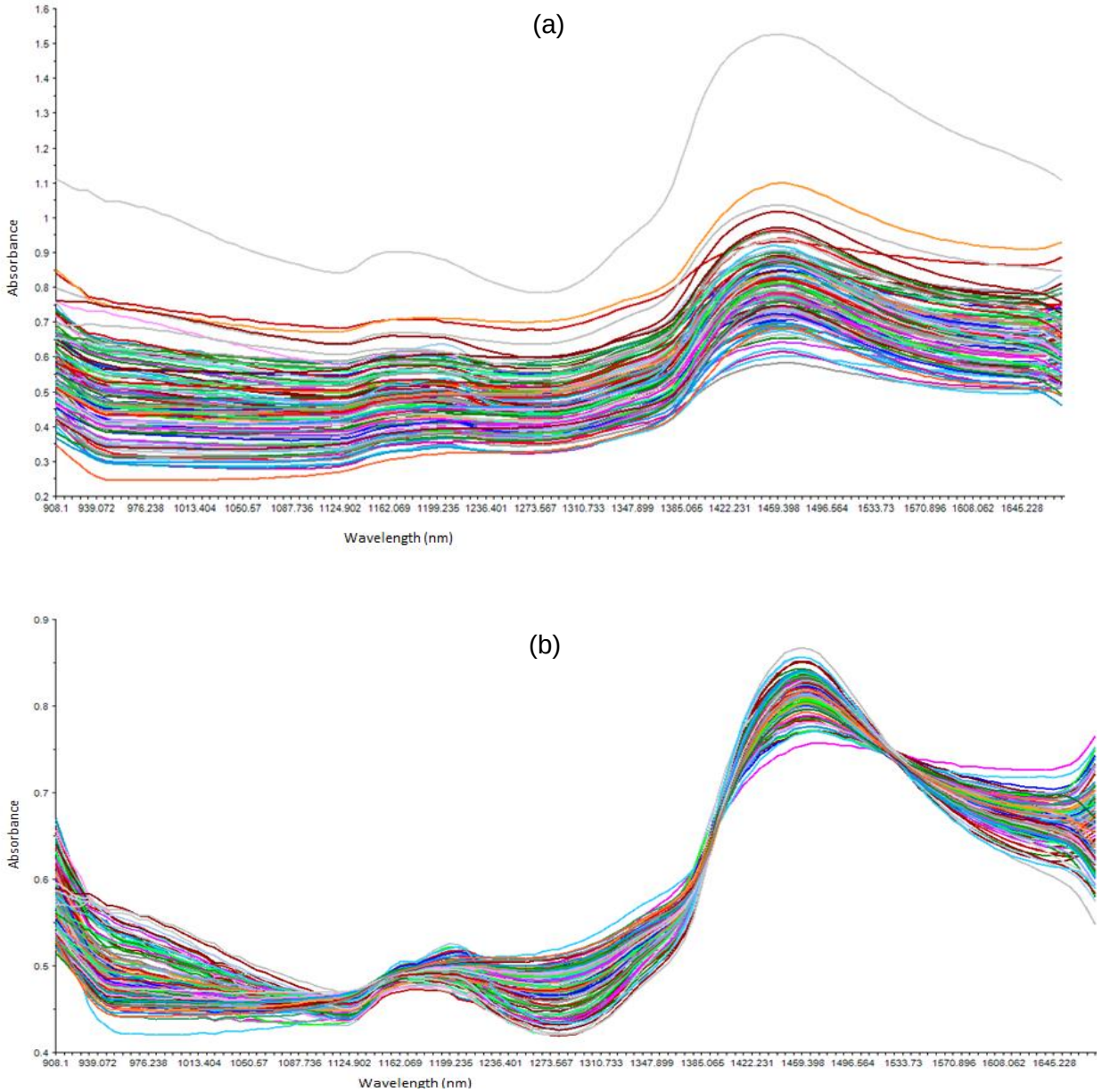


Figure3.5: Spectra of whole fish scanned on the skin with (a) indicating no pre-treatment applied, whereas (b) represent the spectra pre-treated with MSC.

3.3.1.2 Sex

When considering the sex of the fish, an apparent grouping was observed in the PCA score plot of PC 1 vs. PC 2 (Fig. 3.6), capturing 98% of all the variance, as the female samples (blue squares) and the male samples (red circles) formed separate groups. The score plot shows the position of

the two sex groups in the first two PCs. The separation between male and female samples points out the differences in the two sexes. Therefore, PCA was able to group the fish sample set based on different sex groups.

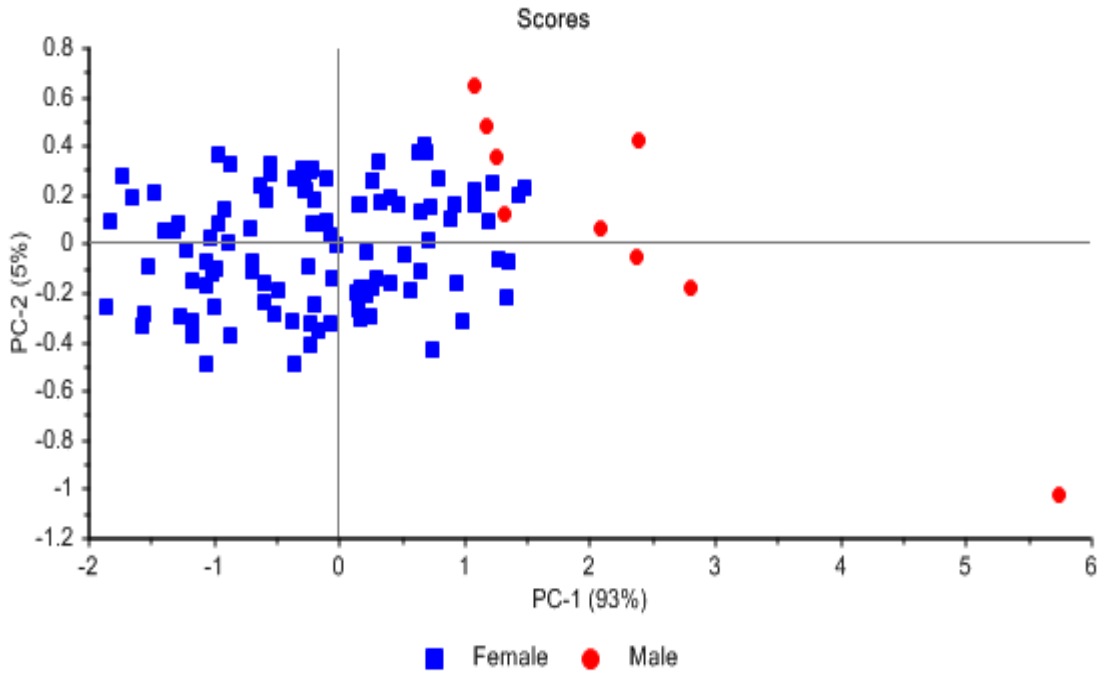


Figure 3.6: PCA score plot of female (blue block) and male (red dots).

3.3.1.3 Fat stage

Although initially seven fat stages were assigned (Van der Lingen & Hutchings, 2005), for the purpose of this PCA model, only three fat stages were used. These three stages were assigned as follows: fat stage one and two as described by Van der Lingen and Hutchings (2005) were assigned as Fat stage 1 in this thesis. Similarly, fat stage three and four were assigned as Fat stage 2, and fat stages five, six and seven were assigned Fat stage 3 based on visual examination of the mesenteric fat. Using the results of the morphophysiological indicators, Fat stage 1, indicating the least fat fish, were coloured in blue squares, Fat stage 2, the intermediate fat stage, was coloured in red circles, whereas the fattiest fish were grouped as Fat stage 3 and coloured in green triangles (Fig. 3.7). As indicated on the score plot of PC 1 vs. PC 2, a slight separation of the different fat stages was evident, with each fat stage forming groups according to their lipid

content. The results provide evidence of lipid reserves of fish, which is considered important for management of fisheries and is also of high practical value for the sardine canning industry

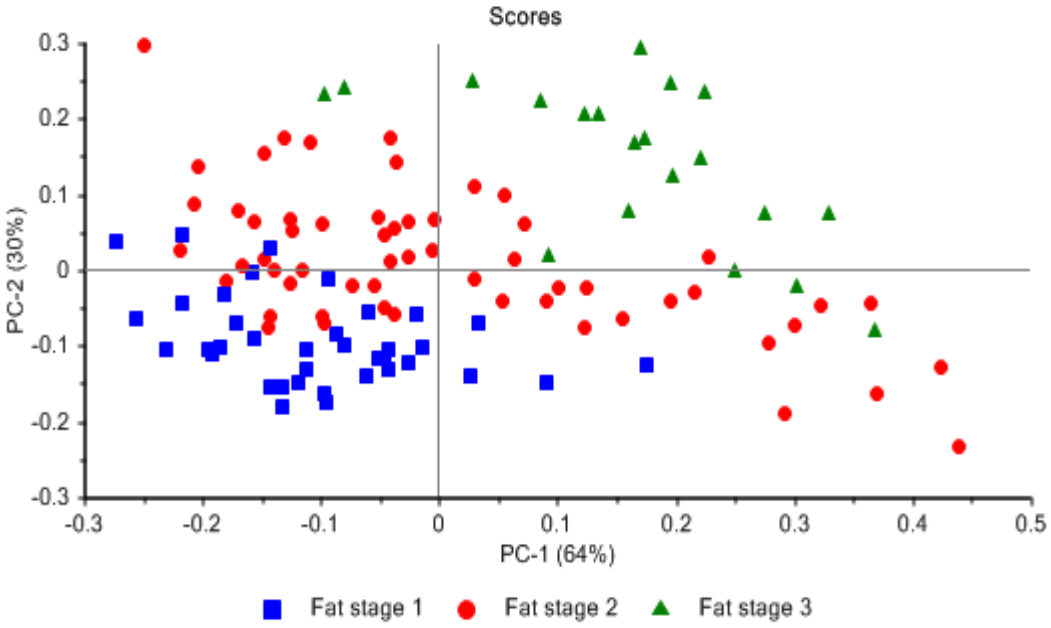


Figure 3.7: A PCA score plot of PC 1 vs. PC 2 (explaining 94% variance in total) coloured according to the extreme fat stages (i.e. fat stage 1, 2 and 3) of the fish sample set.

3.3.1.4 Gonad stage

Using the results of the exploratory stage (PCA modeling), samples were assigned colours according to their gonad stage. Again, only the three extreme stages, i.e. smallest, intermediate and largest gonads (gonad stage 1, 2 and 3), were used. The PCA score plot of PC 1 vs PC 2 showed some clustering. The score plot (Fig. 3.8) shows the position of the gonad stages in the multivariate space of PC1 and PC2. The clear grouping of the gonad stages pointed out the differences in the ovarian state of sardines. Gonad stage 1 (green triangle) was located at the far bottom right of the plot and is characterized by the inactive ovarian state (i.e. stage 1 or 3). Gonad stage 2 (blue squares) was situated closer to stage 3 (red dots) and showed a clear separation in the PCA score plot. It is clearly seen that sardine samples divided into their different gonad stages based on differences in the ovarian state, indicating the different development and maturation stages of the oocyte (Yamamoto, 1956).

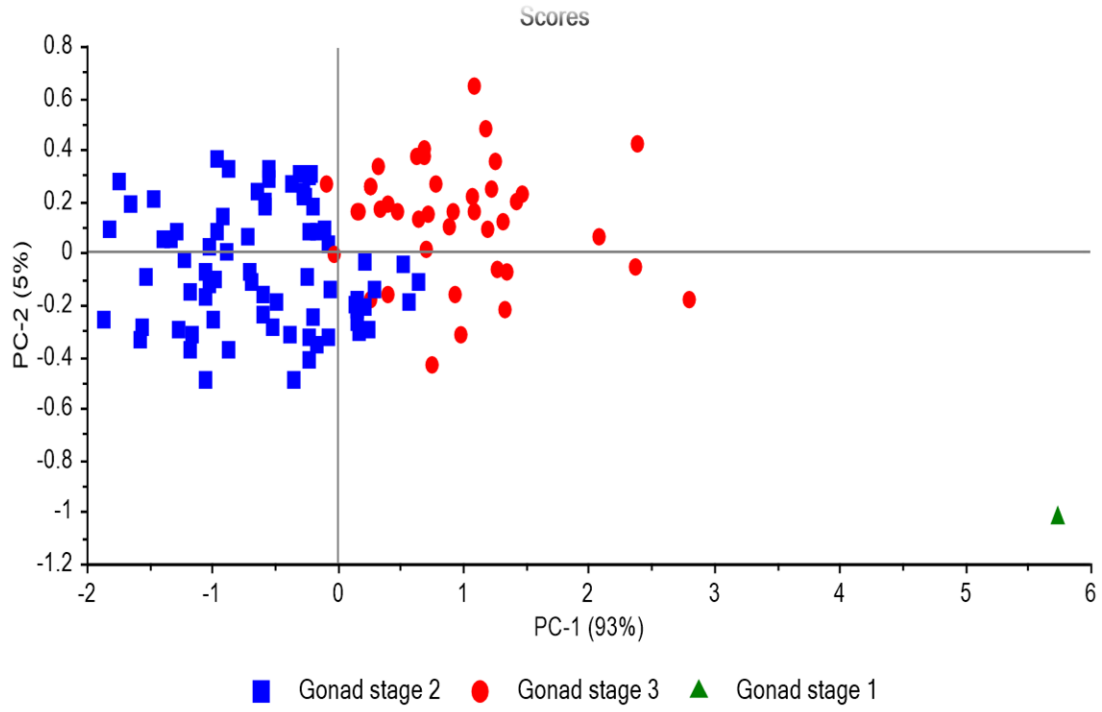


Figure 3. 8: a PCA score plot (PC 1 vs. PC 2) showing some clustering associated with the respective gonad size (i.e. gonad stages 1, 2 and 3, respectively) of the fish samples.

3.3.2 Homogenised sardine samples

3.3.2.1 NIR spectra

Spectra were also acquired from homogenised samples. The mean spectra of the homogenised fish samples are shown in Fig. 3.9. The spectra showed absorption bands around 957 nm, related to H_2O (water), 1 081 nm related to protein and around 1 267 nm related to CH_3 (fat) (Osborne *et al.*, 1993).

For the scans taken of the homogenised samples, all the wavelengths from 908 to 1680 nm were considered. PCA of the spectra was performed using the first seven principal components from the spectral data and was used to examine the possible grouping of samples.

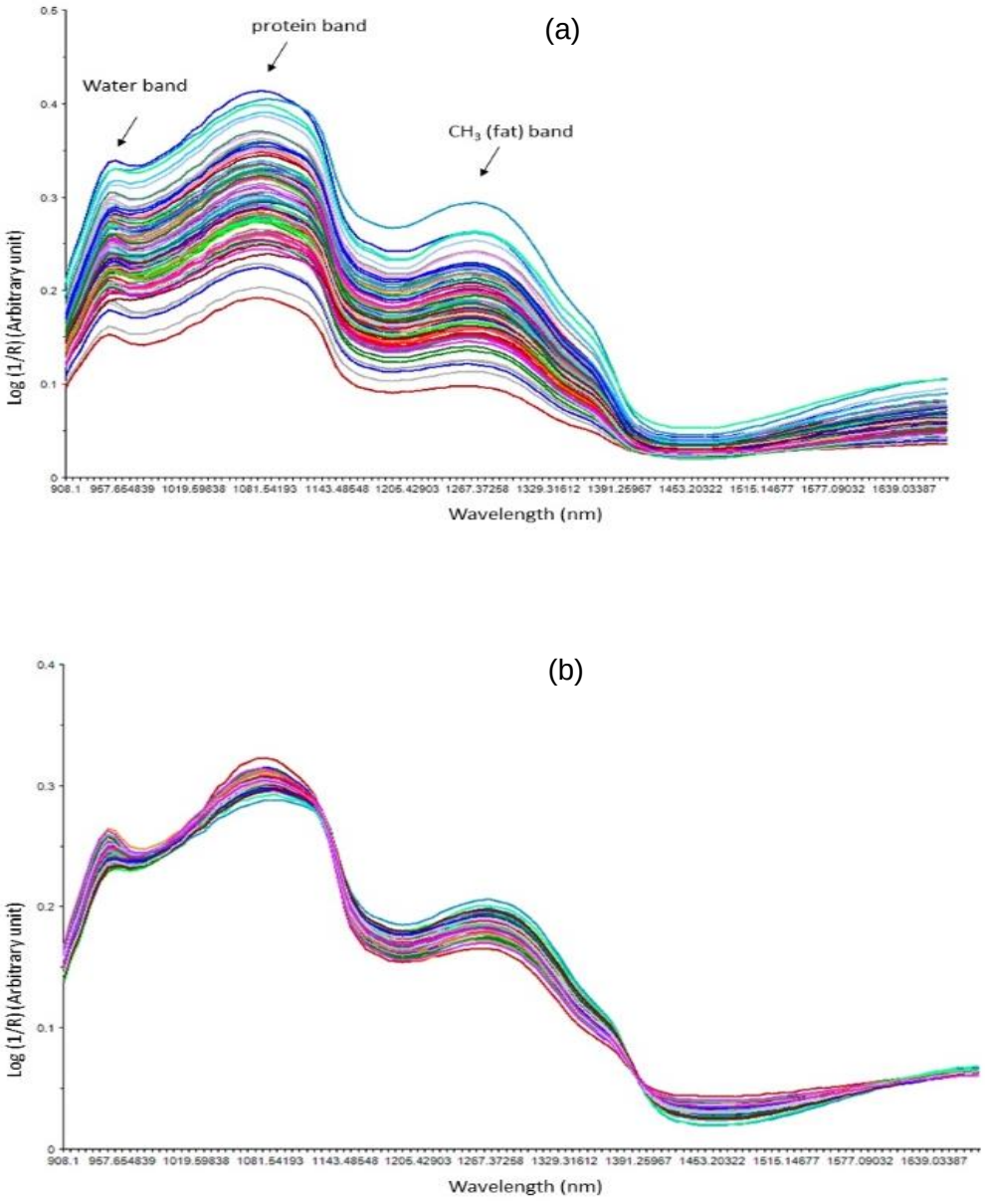


Figure 3. 9: Spectra of homogenised fish samples (a) indicating no pre-treatment application, while (b) indicates MSC pre-treated spectra.

3.3.2.2 Sex

In discriminating between the sex of the homogenised fish samples, there was no evident grouping observed in the PCA score plot of PC 1 vs. PC 2 (Fig. 3.9 a), capturing 96% of all the variance, as the female samples (blue squares) and the male samples (red circles) did not form distinct

clusters. Irrespective of what combination of PCs was investigated, no grouping was observed (combinations of PCs 1 to 7 were considered). Therefore, this fish sample set could not distinguish between male and female fish.

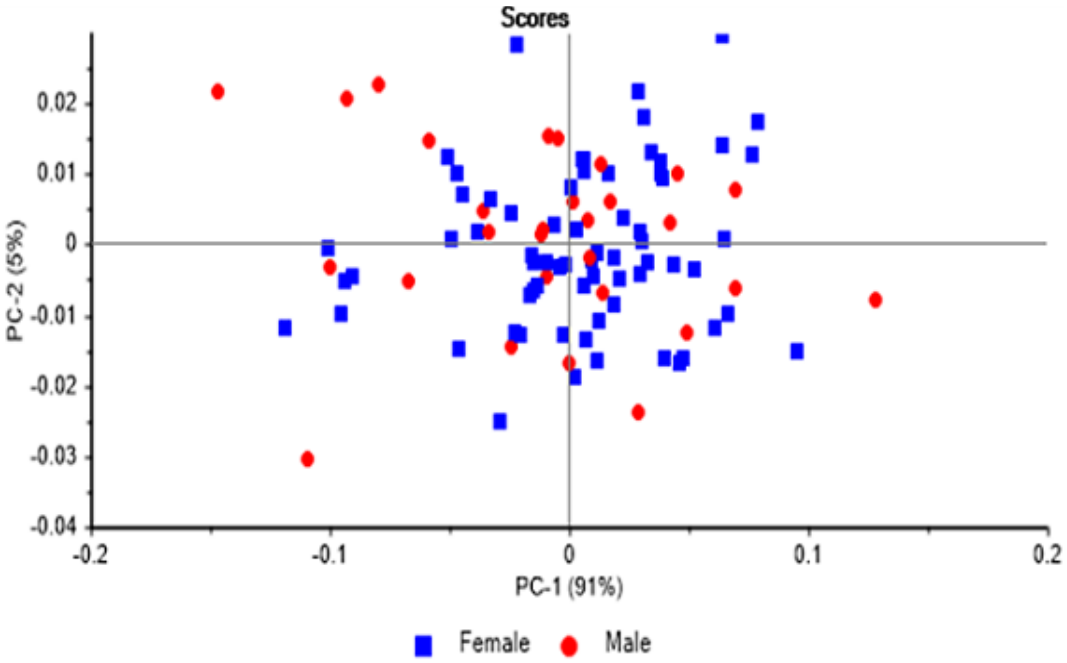


Figure 3. 10: PCA score plot of female (blue blocks) and male (red dots) homogenised fish samples.

3.3.2.3 Fat stage

PCA was performed on the homogenized sample set to determine clustering based on fat stages. Though initially there were seven fat stages identified, for this PCA model, only the two extreme fat stages were considered. Fat stage 1, indicating the least fat fish, were coloured in blue blocks, whereas the fattest fish were grouped as Fat stage 3 and coloured as red dots (Fig. 3.11). As indicated on the score plot of PC 1 vs. PC 2, there is a slight clustering with regards to the visible red dots, grouping the "fatty" fish. The amount of fat in the mesentery depends on the amount of fat associated with both the intestine and stomach (Sinovčič, 1978).

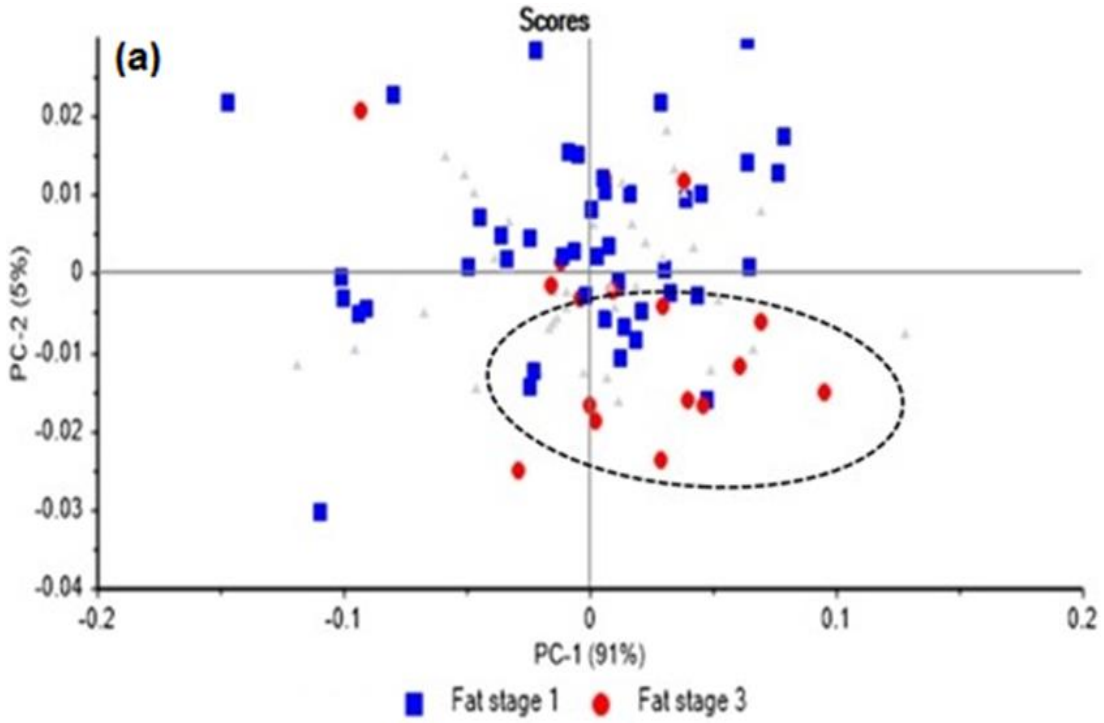


Figure 3. 11: (a) A PCA score plot of PC 1 vs. PC 2 (explaining 96% variance in total coloured according to the respective fat stages of the fish sample set

3.3.2.4 Gonad stage

Using the results of exploratory PCA modeling only the two extreme stages, i.e. smallest and largest gonads were considered. For the PCA score plot (Fig. 3.12), blue squares represented the fish with the smallest gonads, whereas the red dots represented the fish with the largest gonads. The PCA score plot of PC 1 vs. PC 2 showed no grouping. None of the other PC combinations showed any significant grouping. In homogenised samples, the internal organs (viscera) of fish were removed and hence no grouping or clustering was observed.

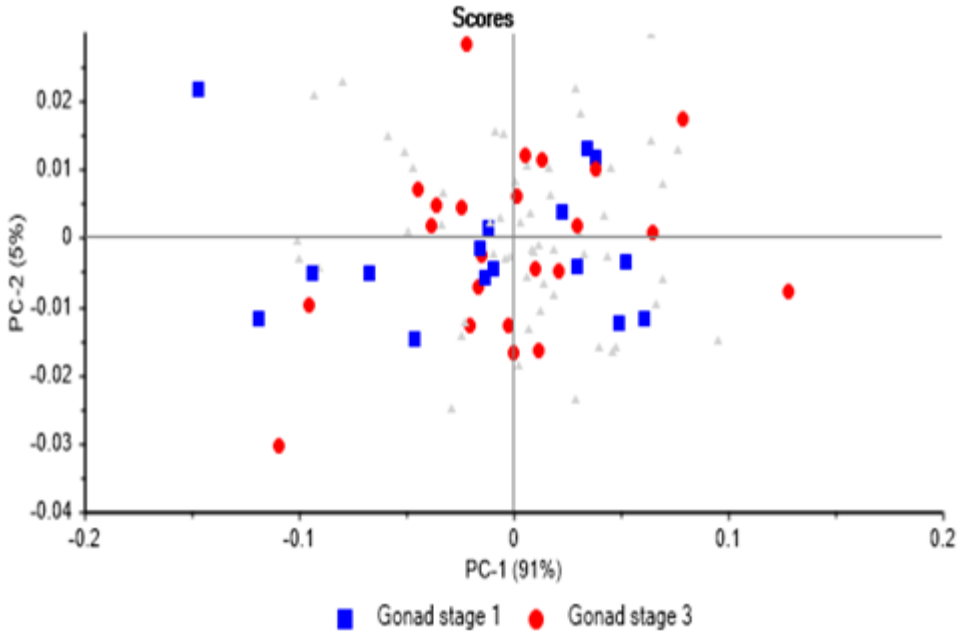


Figure 3. 12: PCA score plot (PC 1 vs. PC 2) (explaining 96% of the variance) coloured according to the gonad stages with stage 1 indicated by the blue squares and stage 3 indicated by the red dots and no apparent clustering present.

3.4 Conclusion

This study demonstrated that sex and fat stages could be used to a fair extent, to segregate the South African whole sardine samples using NIR spectroscopy and PCA. Fat stage grouping was observed using both sample sets of whole fish scanned on their skin, as well as homogenised fish. Sardines with a higher fat content are considered to be in a better condition opposed to sardines with less fat content, thus this grouping property of NIR spectroscopy combined with appropriate chemometrics, can be useful for the canning industry. Also, as segregation of fish according to their gonad stages was seen using whole fish scanned on their skin, the feasibility of NIR spectroscopy was illustrated, being a fast and straightforward method needing no sample preparation. A slight clustering was observed for homogenised fish samples.

The hand-held NIR device furthermore can be used at the receiving dock of a fish factory, making NIR spectroscopy even more convenient. The whole fish sample set was more successful in grouping samples according to their gonad size opposed to the homogenised sample set. Sex, fat stage as well as gonad stage, determined for the respective sample sets as morphophysiological indicators, seemed to be effective for exploratory purposes, especially for

the whole sample set. Therefore, further research that will include a larger representative population of both sexes, as well as sardines representing a varying level of maturity should be investigated.

References

Abaunza P., Murta A.G., Campbel,l N., Cimmaruta, R., Comesaña, A.S., Dahle, G., García Santamaría, M.T., Gordo, L.S., Iversen, S.A, MacKenzie, K., Magoulas, A., Mattiucci, S., Molloy, J., Nascetti, G., Pinto, A.L., Quinta, R., Ramos, P., Sanjuan, A., Santos, A.T., Stransky, C., Zimmerman, C. (2008). Stock identity of horse mackerel (*Trachurus Trachurus*) in the Northeast Atlantic and Mediterranean Sea: integrating the results from different stock identification approaches. *Fisheries Research*, **89**, 196-209.

Abderrazik, W., Baali, A., Schahrakane, Y. & Tazi, O. (2016). Study of reproduction of sardine, *Sardina pilchardus* in the North of Atlantic Moroccan area. *Bioflux*, **9**(3), 507-517.

Agenbag, J. J., Richardson A. J., Demarcq, H., Fréon, P., Weeks, S. & Shillongton, F.A. (2003). Estimating environmental preferences of South African pelagic fish species using catch size- and remote sensing data. *Progress in Oceanography*, **59**, 275-300.

Akkers, T.R., Melo, Y.C. & Veith, W. (1996). Gonad development and spawning frequency of the South African pilchard *Sardinops sagax* during the 1993–1994 spawning season. *South African Journal of Marine Science*, **17**, 183–193.

Anonymous, (2016). Small pelagic fish (sardine, anchovy and round herring). In: *Status of the South African Marine Fishery Resources 2014*. Department of Agriculture, Forestry and Fisheries. Cape Town, South Africa.

Aubourg, S. & Medina, I. (1997). Quality differences assessment in canned sardine (*Sardina pilchardus*) by fluorescence detection. *Journal of Agricultural and Food Chemistry*, **45**, 3617-3621.

Barange, M., Hampton, I. & Roel, B.A. (1999). Trends in the abundance and distribution of anchovy and sardine on the South African continental shelf in the 1990s, deduced from acoustic surveys. *South African Journal of Marine Science*, **21**, 367-391.

Beckley, L.E. & Van der Lingen, C.D. (1999). Biology, fishery and management of sardines (*Sardinops sagax*) in southern African waters. *Marine and Freshwater Research*, **50**(8), 955-978.

Begg, G.A., Waldman, J.R. (1999a). An holistic approach to fish stock identification. *Fisheries Research*, **43**, 35-44.

Begg, G. A., Friedland, K.D. & Pearce, J.B. (1999b). Stock identification and its role in stock assessment and fisheries management: an overview. *Fisheries Research*, **43**, 1-8.

Bianchi, G., Carpenter, K.E., Roux, J.P., Molloy, F.J., Boyer, D.C. & Boyer, H.J. (1999). Field guide to the living marine resources of Namibia. p.265, Rome, FAO.

Blaber, S.J.M., Cyrus, D.P., Albaret, J.J., Chong Ving Ching Day, J.W., Elliott, M., Fonseca, M.S., Hoss, D.E., Orensanz, J., Potter, I.C. & Silvert, W. (2000). Impact of fishing on the structure and functioning of estuarine and nearshore ecosystems. *ICES Journal of Marine Science*, **57**, 558-571.

Bookstein, F.L.; Chernoff, B.; Elder, R.L.; Humphries, J.M.; Smith, G.R. & Strauss, R.E. (1985). Morphometrics in evolutionary biology. *Special Publication,* **15**, Academy of Natural Sciences, Philadelphia.

Bradford, R. G. (1993). Differential utilization of storage lipids and storage proteins by Northwest Atlantic herring (*Clupea harengus harengus*). *Journal of Fish Biology*, **43**, 811–824.

Brosset, P., Fromentin, J., Menard, F., Pernet, F., Bourdeix, J., Bigot, J., Van Beveren, E., Perez, R.M.A., Choy, S. & Saraux, C. (2015). Measurement and analysis of small pelagic fish condition: A suitable method for rapid evaluation in the field. *Journal of Experimental Marine Biology and Ecology*, **462**, 90-97.

Buckworth, R.C. (1998). World fisheries are in crises? We must respond. In: *Reinventing fisheries management* (edited by T.J. Pitcher; P.J.B. Hart & D. Pauly). p.435. Kluwer Academic Publishers, Dordrecht; Boston.

Cadrin, S.X., Friedland, K.D. & Waldman, J.R. (2005). Stock identification methods. In: Applications in fishery science (edited by S.X. Cadrin, K.D. Friedland, J.R. Waldman) Pp. 3-6. San Francisco, Elsevier Academic Press.

Cochrane, K.L., Butterworth, D.S., De Oliveira J.A.A. & Roel, B.A. 1998. Management procedures in a fishery based on highly variable stocks and with conflicting objectives: experiences in the South African pelagic fishery. *Reviews in Fish Biology and Fisheries*, **8**. 177-214.

Coetzee, J.C., Van der Lingen, C.D., Hutchings, L. & Fairweather, T.P. (2008a). Has the fishery contributed to a significant shift in the distribution of South African sardine? *ICES Journal of Marine Science*, **65**, 1676-1688.

Coetzee, J.C, Merkle, D., de Moor, C.L., Twatwa, N.M, Barange, M. & Butterworth, D.S. (2008b). Refined estimates of South African pelagic fish biomass from hydro-acoustic surveys: quantifying the effects of target strength, signal attenuation and receiver saturation. *African Journal of Marine Science*, **30**, 205-217.

Coetzee, J.C., de Moor, C.L. & Butterworth, D.S. (2018). A summary of the South African sardine (and anchovy) fishery. *MARAM/IWS/2018/Sardine/BG1*.

Corti, M., Thorpe, R.S., Sola, L., Sbordoni, V. & Cataudella, S. (1988). Multivariate morphometrics in aquaculture: A case study of six stocks of the Common Carp (*Cyprinus carpio*) from Italy. *Canadian Journal of Fisheries and Aquatic Sciences*, **45**, 1548-1554.

Costa, A.M. (2009). Macroscopic vs microscopic identification of the maturity stages of female horse mackerel. *ICES Journal of Marine Science*, **66**, 509-516.

Coyle, T. (1998). Stock identification and fisheries management: the importance of using several methods in a stock identification study. In: *Taking stock: defining and managing shared resources* (edited by D.A. Hancock). Pp. 173-182, Australian Society for Fishery Biology, Sydney.

Cozzolino, D., Chree, A., Scaife, J.R. & Murray, I. (2005). Usefulness of near-infrared reflectance (NIR) spectroscopy and chemometrics to discriminate fishmeal batches made with different fish species. *Journal of Agricultural and Food Chemistry*, **53**, 4459-4463.

Crawford R.J.M., Sabarroa, P.S., Fairweather, T.P., Underhill, L.G. (2008). Implications for seabirds off South Africa of a long-term change in the distribution of sardine. *African Journal of Marine Science*, **30**(1), 177-184.

Culley, M. (1971). The pilchard: biology and exploitation. *International Series of Monographs on Pure and Applied Biology Zoology Division*. Pp. 241. Pergamon Press, Oxford.

Department of Agriculture, Forestry and Fisheries, (2011/12). Annual Report. [WWWdocument]URLhttp://www.nda.agric.za/docs/AnnualReports/2011_12/AR2012.pdf. 28 April 2018.

De Goede, J. (2004). Spatial and temporal variability in lipid content and gonadosomatic index of sardine (*Sardinops sagax*) in Southern Benguela ecosystem. Master of Technology Thesis. Cape Technikon. South Africa.

De Graaf, G. & Gabribaldi, L., 2014. The value of African fisheries, FAO circular No. 1093. Pp. 76. Rome.

De Moor, C.L. & Butterworth, D.S. (2015). Assessing the South African sardine resource: two stocks rather than one? *African Journal of Marine Science*, **37**, 41–51.

Dörücü, M. (2000). Changes in protein and lipid content of muscle, liver and ovaries in relation of Diphyllobothrium spp. (Cestoda) infection in Powan (*Coregonus lavaretus*) from Loch Lomond, Scotland. *Turkish Journal of Zoloogy*, **24**, 211-218.

Fairweather, T. P., Hara, M., Van der Lingen, C. D., Raajær, J., Shannon, L. J., Louw, G. G., DegnbolGNBOL, P. & Crawford, R. J. M. (2006) A knowledge base for management of the

capital-intensive fishery for small pelagic fish off South Africa. *African Journal of Marine Science*, **28**, 645–660.

Favero, G.C., Gimbo, R.Y., Montoya, L.N.F., Zanuzzo, F.S. & Urbinati, E.C. (2017). Fasting and refeeding lead to more efficient growth in lean pacu (Piaractus mesopotamicus). *Aquaculture Research*, **49**, 359-366.

Fontoura, N.F., Braun, A.S. & Milani, C.C. (2009). Estimating size at first maturity (L_{50}) from Gonadosomatic Index (GSI) data. *Neotropical Ichthyology*, **7**(2), 217-222.

Galindez, E.J., Andrade, M.D. & Estecondo, S. (2014). Morphological indicators of initial reproductive commitment in *Mustelus schmitti* (Springer, 1939), Chondrich thyes, Triakidae): folliculogenesis and ovarian structure over the life cycle. *Brazillian Journal of Biology*, **74**(3), 1399-1413.

Garcia, S.M. & Newton, C. (1997). Current situation, trends and prospects in catch fisheries. In: *Global trends in fisheries management*. Pp. 3-27. American Fisheries Society Symposium. Bethesda.

Garthwaite, T. (2010). Fish quality. In: *Fish Canning Handbook* (edited by L. Brett). Pp. 102-130. Blackwell Publishing, United Kingdom.

George, K.C. (1959). A method for distinguishing the sex of the oil-sardine Sardinela longiceps Val. In the field. *Indian Journal of Fisheries*, **6**(2), 322-326.

Grant, W.S., Clark, A.M. and Bowen, B.W. (1998). Why restriction length polymorphism analysis of mitochondrial DNA failed to resolve sardine (*Sardinops*) biogeography: insights from mitochondrial DNA cyctochrome b sequences. *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 2539-2547.

Grant, W.S., Bowen, B.W. (1998). Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity*, **89**,415-426.

Gulland, J.A. (1965). Estimation of mortality rates. Annex to Report of the Arctic Fisheries Working Group. Report No. ICES CM 1965(3). International Council for the Exploration of the Seas, Copenhagen.

Hall, S.J. (1999). The effects of fishing on marine ecosystems and communities. Blackwell Science, Great Britain.

Hampton, I. (1992). The role of acoustic surveys in the assessment of pelagic fish resources on the South African continental shelf. In: B*enguela trophic functioning* (edited by A.I.L.

Payne, K.H. Brink, K.H. Mann, R. Hilborn). *South African Journal of Marine Science*, **12**, 1031-1050.

Hampton, K.A., Cavinato, A.G., Mayes, D.M., Boe, S.J. & Hoffnagle, T.L. (2002). Near infrared spectroscopic classification of gender and maturity in Chinook salmon (*Oncorhynchus tshawytscha)*. *Eastern Oregon Science Journal*, **17**, 31-36.

Hara, M., Greenberg, S., Thow, A., Chimatiro, S. & Toit, A. (2017). Trade and investment in fish and fish products between South Africa and the rest of SADC: Implications for food and nutrition security. *Working Paper 47*. PLAAS, UWC: Cape Town.

Hayes, J.P. & Shonkwiler, J.S. (2001). Morphometric indicators of body condition: worthwhile or wishful thinking? In: *Body Composition Analysis of Animals* (edited by J.R. Speakman). Pp. 8–38. Cambridge University Press, Cambridge: UK.

Hussey, N.E., Cocks, D.T., Dudley, S.F.J., McCarthy, I.D. & Wintner, S.P. (2009). The condition conundrum: application of multiple condition indices to the dusky shark *Carcharhinis obscurus*, *Marine Ecology Progress Series*, **380**, 199-212.

Idris, I., Moloney, C.L. & van der Lingen, C.D. (2016). Spatial variability in branchial basket meristics and morphology of South African sardine *Sardinops sagax*. *African Journal of Marine Sciences*, **38**(3), 351-362.

Isaacs, M. (2016). The humble sardine (small pelagics): fish as food or fodder. *Agriculture & Food Security*, **5**(27), 1-14.

Jolliffe, I.T & Cadima, J. (2016). Principal component analysis: a review and recent developments. *Philosophical Transactions A*, **374**, 1-16.

Jones R.E., Petrell, R.J., Pauly D. (1999) Using modified length-weight relationships to assess the condition of fish. *Aquacultural Engineering*, **20**, 261-276.

Khajuria, B., Langer, S. & Sharma, R. (2014). Length weight relationship and condition of Tor putitora from Jhajjar stream. *International Journal of Scientific & Engineering Research*, **5**(2), 567-572.

Knapp, J.M., Aranda, G., Medina, A. & Lutcavage, M. (2014). Comparative assessment of the reproductive status of female Atlantic Bluefin tuna from the Gulf of Mexico and the Mediterranean Sea. *PLos One*, **9**(6), 1-9.

Kocovsky, P.M., Adams, J.V. & Bronte, C.R. (2009). The effect of sample size on the stability of principal components analysis of truss-based fish morphometrics. *Transactions of the American Fisheries Society*, **138**, 487-496.

Krainer, Van der Lingen, C.D. & Fréon, P. (2001). A comparison of condition factor and gonadosomatic index of sardine *Sardinops sagax* stocks in the northern and southern

Benguela upwelling ecosystems, 1984-1999. *South African Journal of Marine Science*, **23**, 123-134.

Lambert, Y., Dutil, J.-D., (1997). Can simple condition indices be used to monitor and quantify seasonal changes in the energy reserves of cod (*Gadus morhua*)? *Canadian Journal of Fish Aquatic Science*, **54**, 104–112.

Larsen, E., Jemsen, S. & Zappey, H. (year?) Quality management and measurement in the fish industry. In: *Seafood from producer to consumer, integrated approach to quality* (edited by J.B. Luten, T. Børresen & J. Oehlenschläger. Pp. 403-410. Amsterdam, The Netherlands: Elsesivier Sience

Lett, C., Veitch, J., Van der Lingen, C.D., Hutchings, L. (2007). Assessment of an environmental barrier to transport of ichthyoplankton from the southern to the northern Benguela ecosystems. *Marine Ecology Progress Series*, **347**, 247-259.

Levêque, C. (1995). Role and consequences of fish diversity in the functioning of African freshwater ecosystems: a review. *Aquatic Living Resources*, **8**, 59-78.

Lloret, J., Gil de Sola, L., Souplet, A & Galzin, R. (2002). Effects of large scale habitat variability on condition of dermesal exploited fish in the north-western Mediterranean. *ICES Journal of Marine Science*, **59**, 1215-1227.

Lloret, J., Shulman, G. & Love, R.M. (2014). Description of condition indicators. In: *Condition and Health Indicators of Exploited Marine Fishes*. Chicheste, USA: John Wiley & Sons Ltd.

Lu, X., Webb, M., Talbott, M., Van Eenennaam, J., Palumbo, A., Linare-Casenave, J., Doroshov, S., Struffeneffer, P. & Rasco, B. (2010). Distinguishing ovarian maturity of farmed white sturgeon (*Acipenser transmontanus*) by Fourier Transform Infrared Spectroscopy: A potential tool for caviar production management. *Journal of Food Chemistry & Agriculture*, **58**, 4056-4064.

Matthews, J.P. (1964). The pilchard of southwest Africa (*Sardinops occellata*), sexual development, condition factor and reproduction, 1957-1960. *Administration of South West Africa Marine Research Laboratory Investigational Rep*, **10**, 96.

McPherson, L.R., Slotte, A., Kvamme, C., Meier, S. & Marshall, C.T. (2011). Inconsistencies in measurement of fish condition: a comparison of four indices of fat reserves for Atlantic herring (*Clupea herengus*). *ICES Journal of Marine Science*, **68**(1), 52-60.

Mesías, M., Holgado, F., Sevenich, R., Briand, J.C., Márquez-Ruiz, G & Morales, F.J. (2015). Fatty acid profile in canned tuna and sardine after retort sterilisation and high pressure thermal sterilisation treatment. *Journal of Food and Nutrition Research*, **54**(2), 171-178.

Mojekwu, T.O. & Anumudu, C.I. (2015). Advanced techniques for morphometric analysis in fish. *Journal of Aquaculture research & Development*, **6**(8), 1-6.

Morgan, M.J. (2004). The relationship between fish condition and the probability of being mature in American plaice (*Hippoglossoides platessoides*). *Journal of Marine Science*, **61**(1), 64-70.

Morimoto, H. (1996). Effects of martenal nutritional conditions on number, size and lipid content of hydrated eggs in Japanese sardine from Tosa Bay, south western Japan. In; *Survival Strategies in Early Life Stages of Marine Resources* (edited by Y. Walanabe, Y. Yamashita, & Y. Oozeki. Pp. 3-12. AA Balkema: Rottedam.

Murphy, B.R., Brown, M.L. & Springer, T.A. (1990). Evaluation of the relative weight (W_r) index with new application to Walleye. *North American Journal of Fisheries Management*, **10**, 85-97.

Murua H, Kraus G., Saborido-Rey, F., Witthames, P.R, Thorsen, A., Junquera, S. (2003). Procedure to estimate fecundity of marine fish species in relation to their reproductive strategy. Journal of Northwest Atlantic Fishery Science, **33** 33–54.

Murua H, Saborido-Rey, F. (2003). Female reproductive strategies of marine fishes of the North Atlantic. Journal of Northwest Atlantic Fishery Science, **33**, 23–31.

Mustać, B. & Sinovčić, G. (2009). Comparison of mesenteric and tissue fat in relation to sexual cycle of the sardine, *Sardina pilcardus* (Walbaum, 1972) in the eastern Middle Adriatic fishery grounds (Croatia). *Journal of Applied Ichthyology*, **25**, 595-599.

Mustać, B. & Sinovčić, G. (2010). Reproduction, length-weight relationship and condition of sardine, *Sardina pilchardus* (Walbaum, 1972), in the eastern Middle Adriatic Sea (Croatia). *Periodicum Biologorum*, **112**(2), 133-138.

Newman, G.G. (1970a). Stock assessment of the pilchard *Sardinops ocellata* at Walvis Bay, South West Africa. *Investigational Report, Division of Sea Fisheries South Africa*, **85**, 1–13.

Newman, G.G. (1970b). Migration of the pilchard *Sardinops ocellata* in Southern Africa. *Investigational Report, Division of Sea Fisheries South Africa*, **86**, 1–6.

Osborne, B.G., Fearn, T. & Hindle, P.H. (1993). Practical NIR Spectroscopy with applications in food and beverage analysis, 2nd edition, Pp. 1-220. England: Longman Group UK Limited.

Pořízka, P., Klus, J., Képeš, E., Prochazka, D., Hahn, D.W. & Kaiser, J. (2018). On the utilization of principal component analysis in laser-induced breakdown spectroscopy data analysis, a review. *Spectrochimica Acta Part B: Atomic Spectroscopy*, **148**, 65-82.

Schamber, J.L., Esler, D. & Flint, P.L. (2009). Evaluating the validity of using unverified indices of body condition. *Journal of Avian Biology*, **40**, 49-56.

Schulte-Hostedde, A.L., Millar, J.S. & Hickling, G.J. (2001). Evaluating body condition in small mammals. *Canadian Journal of Zoology*, **79**, 1021-1029.

Schulein, F.H., Boyd, A.J. & Underhill, L.G. (1995). Oil-to meal ratios of pelagic fish taken from northern Benguela systems: seasonal patterns and temporal trends, 1951-1993. *South African Journal of Marine Science*, **15**, 61-82.

Shulman, G.E. Love, R.M. (1999). Indicators of fish condition. In: *Advances in Marine Biology: The Biochemical Ecology of Marine Fishes* Volume 36 (edited by A.J. Southward, P.A. Tyler & C.M. Young). Pp. 205-206. California, USA: Academic Press Limited.

Simon, C.J., Rodemann, T., Carter, C.G. (2016). Near-infrared spectroscopy as a novel non-invasive tool to assess spiny lobster nutritional condition. *PLoS ONE*, **11**(7), 1-15.

Simpson, A.C. (1982). A review of the data based on tropical multispecies stocks in the south east Asian region. In: *Theory and Management of Tropical Fisheries* (edited by D. Pauly & G.L. Murphy). Pp. 5-32, Cronulla, Australa.

Sinovčić, G. (1978). On the ecology of anchovy *Engraulis encrasicolus* (L.), in the central Adriatic. Acta Adriat. 19(2), 32.

Sreelekshmi, S., Benno Pereira, F.G. & Binil, V.S. (2017). Morphometric evaluation of an indigenous fish belonging to genus Devario from three river systems of South Kerala. *Journal of Biodiversity and Environmental Sciences*, **10**(2) 106-114.

Van der Lingen, C.D. & Durholtz, M.D. (2003). Review of variability in biomass, growth, and other characteristics of anchovy *Engraulis encrasicolus* and sardine *Sardinops sagax* in the Benguela Current upwelling system. Unpublished report.

Van der Lingen, C.D, Shannon, L.J, Cury, P., Kreiner, A., Moloney, C.L, Roux, J.P. & Vaz-Velho, F. (2006). Resource and ecosystem variability, including regime shifts, in the Benguela Current system. *Large Marine Ecosystem*. **14**,147–185

Van der Lingen, C.D. & Hutchings, L. (2005). Estimating the lipid content of pelagic fish in the southern Benguela by visual assessment of their mesenteric fat. *African Journal of Marine Science*, **27**(1), 45-53.

Vélez-Arellano, N., Garciá-Domínguez, F.A., Lluch-Cota, D.B., Gutiérrez-González, J.L., Salcido-Guevara, L.A. & Sanchez-Gardenas, R. (2016). Morphophysiological indices of the gren abalone *Haliotis fulgens* Philippi, 1845 at Mexican ocean pacific coast. *Turkish Journal of Fisheries and Aquatic Sciences*, **17**, 31-39.

Waldman, J.R. (2005). Definition of stocks: an evolving concept. In: *Stock identification methods*: *Applications in fisheries science* (edited by S.X. Cadrin, K.D. Friedland & J.R. Waldman). Pp 7-1 6. San Francisco, Elsevier Academic Press.

Waples, R.S. (1998). Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, **89**, 438-450.

Whitehead, P.J.P. (1985). An annoted and illustrated catalogue of the herrings, Sardines, pilchards, sprats, shads, anchovies and wolf herrings. Part 1, Chirocentridae, Clupeidae and Pristigasteridae. Clupeoid fishes of the world, Vol. 7, FAO species catalogue.UK.

WWF. (2014). From boat to plate: Linking the seafood consumer and supply chain.[WWWdocument].URL:http://awsassets.wwf.org.za/downloads/wwfsassi_boattoplate_web.pdf. Accessed 28 April 2018.

Yamamoto, K. (1956). Studies on the formation of fish eggs: I. Annual cycle in the development of ovarian eggs in the flounder*, Liopsetta obscura*. *Journal of the Faculty of Science*, **12**(3): 362-376.

Zlatanos, S. & Lastaridis, K. (2007). Seasonal variation in the fatty acid composition of three Mediterranean fish – sardine *(Sardina pilchardus*), anchovy (*Engraulis encrasicholus*) and picarel (*Spicara smaris*). *Food Chemistry*, **103**, 725-728.

Zorica, B. & Keč, V. Č. (2012). Age, growth and mortality of the garfish, *Belone belone* (L 1761) in the Adriatic Sea. *Journal of the Marine Biological Association of the United Kingdom*, **93**(2), 365-372.

4 CHAPTER FOUR

PREDICTING THE PROXIMATE COMPOSITION OF SOUTH AFRICAN SARDINES, USING NEAR INFRARED (NIR) SPECTROSCOPY AND PARTIAL LEAST SQUARES (PLS) REGRESSION MODELLING

Abstract

Quality and safety of fish are investigated and recognised as an important issue globally. Consumers are expecting manufacturers to provide high-quality fish products. All these factors have underlined the need to develop cost-effective technologies for the rapid measurements of the proximate composition of sardines, especially for quality control purposes in the fish canning industry. Successful NIR prediction models enable fast, low-cost and relatively simple measurements opposed to tedious conventional methods. The moisture, ash, fat and protein content (proximate composition) of South African (SA) sardines (*Sardinops sagax ocellatus*) were determined using conventional analytical methods. These methods included oven-drying in determining the percentage (%) moisture, incineration in a muffle furnace for the % ash, solvent extraction method for fat % determinations, and lastly the Dumas combustion (Leco analyser) method to determine the % protein. Additionally, the usefulness of near infrared (NIR) spectroscopy was investigated as an alternative method for proximate composition prediction of the respective fish samples. NIR spectra were acquired from homogenised samples, using a compact hand-held device utilising the wavelength range of 908 to 1650 nm. The proximate measurements, as obtained by the conventional methods, were used as reference values for the construction of PLS regression models. Spectra were pre-treated with multiplicative scatter correction (MSC), as well as second derivative Savitzky-Golay smoothing. Prediction statistics for the respective sample sets were: R^2_v = 0.22, SEP = 3.13%, RPD 1.15 for moisture, R^2_v = 0.24, SEP = 0.056%, RPD 1.07 for ash, R^2_v = 0.61, SEP = 2.46%, RPD 1.47 for fat and R^2_v = 0.22, SEP = 3.41%, RPD 1.07 for protein. These results indicated that reasonable predictions could not be made using NIR spectroscopy.

4.1 Introduction

In a world where over 70% of the planet is covered with water, fish products represent an essential component of the global food intake to improve the nutrition, health, and well-being of all people (Tacon & Metian, 2013). The recognition of fish as food of high digestibility and nutritive value for human consumption dates back to as early as 1918 (Geiger & Borgstrom, 1962). Fish meat is an excellent source of essential amino acids and a good source of nutrients and is also easily digestible because it contains long muscle fibres (YÁÑEZ *et al*., 1976; Capanio *et al*., 2004; Pirestani *et al*., 2009; Begum & Mina, 2012).

In general, fish consists of protein (10 to 22%), fat (0.2 to 25%), minerals (0.5 to 5%), and water (65 to 90%) (Azim *et al*., 2012; Bagthasingh *et al*., 2016). These components vary in quantity and nature from species to species (as can be seen by the large ranges in which they are found) depending on age, sex, environment, season, organs and muscle location being analysed (Huss, 1988; Khitouni *et al*., 2014; Bagthasingh *et al*., 2016). Furthermore, the differences in the proximate composition of fish are closely associated with their feed intake, and variations in the lipid content are much larger than that of protein and minerals (Bagthasingh *et al*., 2016).

Fish is a highly perishable food commodity because of its high moisture and nutrient content. The relatively high moisture content in fish justifies its perishability either during storage or processing (Mota da Silva *et al*., 2008; Madibbo *et al*., 2014). Water in fish tissues exists in three different forms, i.e. free, adsorbed and bound water (Sen, 2005; Mota da Silva *et al*., 2008). In seafood, moisture content is very crucial since it affects the organoleptic quality, microbiological stability, physical characteristics and shelf life (Sen, 2005; Mota da Silva *et al*., 2008).

Protein is the largest quantity of dry matter in fish, providing about 16% of the protein consumed by the world's population (Bagthasingh *et al*., 2016). Fish proteins are essential in fish due to their high nutritional quality concerning their balanced amino acid composition (Sen, 2005). Skeletal muscle proteins of fish may be distinguished as either (i) sarcoplasmic, (ii) myofibrilla and (iii) stroma proteins (Sen, 2005).

Fats (lipids) provide energy reserves and are essential components of fish muscle (Bagthasingh *et al*., 2016). When lipid levels exceed 1% in a body region, that region can be classified as being a fat depot (Bagthasingh *et al*., 2016; Huss, 1988). Fish comprising a fat content as low as 0.5% and as high as 16-18% are common (Bagthasingh *et al*., 2016). In many fish species, lipids build up during the feeding season and decrease during spawning. Such fish show significant variations in lipid with season and sexual maturity (Bagthasingh *et al*., 2016). Certain fish species have been found to show an increase in lipid content as their size increases (Huss,

1988), with fatty fish like sardines showing apparent variations in the lipid content after reaching maturity (Jacquot, 1961).

Fish tissue is an excellent source of macro and essential trace elements such as iron (Fe), zinc (Zn) and selenium (Se) and usually contains these elements in small quantities. Some of these trace elements are constituents of many enzyme systems, metabolic mechanisms, and as such, contribute to the growth of the fish (Briggs & Schweigert, 1990; Adewumi *et al*., 2014). Data on fish chemical composition is critical since its nutritive value is reflected in its biochemical content and indicates the fish quality (Simhachalam *et al*., 2015).

Conventional chemical analysis methods are used to characterise and quantify the composition of fish, but the main disadvantages of these methods are that they are usually costly, destructive and labour intense (Khodabux *et al*., 2007). Conventional methods also necessitate a significant number of animals to be sacrificed to obtain a representative sample for analysis (Rasco *et al*., 1991). Thus, there has been increasing use of near-infrared (NIR) spectroscopy for quantitative analysis of foodstuffs, in combination with advances in chemometrics (Baeten & Dardenne, 2002; Belton, 1997; Jhaumeer-Laullo & Ramasami, 2005).

The main advantages of NIR spectroscopy are that it is a rapid and non-destructive method of analysis (Baeten & Dardenne, 2002; Osborne, 2002). Issakson *et al*. (1995) published results of NIR spectroscopy predictions of the proximate analysis of whole, as well as minced Atlantic salmons. Similarly, a study (on whole salmons) by Downey (1996) using NIR spectroscopy for the predictions of the proximate composition indicated good predictions. When NIR spectroscopy was applied on sea bass, also to predict the proximate composition thereof, better results were found for the minced samples compared to those from scans of the whole fish (Xicatto *et al*., 2004).

A conventional method to facilitate predictions from a large set of independent variables to a set of dependent variables, is that of partial least squares (PLS) regression. It involves the projection of complex NIR data into analytical parameters (Garcia-Jares & Medina, 1997; Dahlman, 2012).

The study aimed to test the ability of NIR spectroscopy in combination with PLS regression models to predict the proximate composition of South African sardines, using measurements acquired by conventional methods as reference values.

4.2 Materials and methods

4.2.1 Samples

Sardine samples were procured along the West Coast of South Africa from commercial purse seiners (fishing vessels). These fish samples were obtained from the Department of Agriculture, Forestry and Fisheries (DAFF), Cape Town, South Africa. There were two fishing dates: i.e. 28 June 2016 and 3 July 2017 with separate locations (Station A32578 Grid 4705A on 28 June and Station A32598 Grid 55-01 on 3 July) for fishing. Once caught, the fish were covered with blue polyethylene bags and immediately frozen on board of the respective ships. The fish samples were kept frozen and transported to the laboratories of the Department of Food Science and Technology (DFST), Cape Peninsula University of Technology (CPUT) at their (DAFF's) earliest convenience. At DFST the samples were vacuum packed and stored in a freezer between -17 and -20°C until subjected to NIR spectral acquisition and proximate analysis

4.2.2 Moisture content

The moisture content of each respective sample was determined using 3 g homogenised sample. Moisture content was determined in duplicate for each sample. The method involved a drying period of 3 h at 100°C in an SVAC4 SHEL LAB, 4.5 Cu.Ft (125 L) vacuum oven (DAIGGER & COMPANY, Vernon Hills, United States). The official method for moisture content determination as described in the Association of Official Analytical Chemists (AOAC) method 934.01 (AOAC, 2012) was followed. The samples were heated under the specified conditions, and the weight loss was used to calculate the moisture content of the samples using the following equation:

$$\text{Moisture } \% = \frac{\text{Weight of wet sample} - \text{weight of dry sample}}{\text{sample weight}} \times 100 \qquad \text{Equation 4.1}$$

4.2.3 Ash content

For all the respective samples, the ash content (dry weight basis) was determined on the moisture-free samples. These determinations were also done in duplicate, and the official AOAC method 942.05 was used (AOAC, 2002). This method involved the incineration of the respective samples for 6 hours at 500°C in a top opening XLH-1200BF-2 muffle furnace (Zhengzhou Sheraho Lab Equipment Co, Ltd). The following equation was used to calculate the ash content:

$$\text{Ash } \% \text{ (as is basis)} = \frac{\text{weight after ashing} - \text{weight of crucible}}{\text{original sample weight} + \text{weight of crucible}} \times 100$$

4.2.4 Fat content

The total fat (or lipid) content of the respective fish samples was determined using 3 g homogenised samples, again performed in duplicate. The method involved the gravimetric extraction of chloroform/methanol using Buchner funnel as described by Lee *et al.* (1996). As the samples were expected to contain more than 5% fat, a chloroform/methanol solution concentration of 2:1 (v/v) was used (Lee, 1996). The following equation was used to calculate the fat content:

$$\text{Fat } \% = \left(\frac{(\text{fat beaker+fat}) - \text{fat beaker})}{\text{Sample weight}} \div \frac{\text{chloroform volume}}{3}\right) \times 100$$

4.2.5 Protein content

The total crude protein (% wet weight) of the defatted, dried and ground fish samples was analysed in duplicate using the Dumas combustion method 992.15 (AOAC, 2002). The samples (0.15 g) were encapsulated in a Leco™ foil sheet and analysed in a Leco Nitrogen/Protein analyser (LECO Corporation, Saint Joseph, Michigan, USA). The Leco analyser was calibrated with ethylene-diamine-tetra-acetic acid (EDTA) before the analyses of each sample. The results were obtained as percentage nitrogen (% N), which was then converted to total percentage crude protein by multiplying the nitrogen value with a conversion factor of 6.25. This was then converted to percentage protein per gram of fish sample by using the following equation:

$$\text{Protein } \% = \text{N x } 6.25 \qquad \text{Equation 4.4}$$

$$\text{Where N} = \text{Leco N value} \times \frac{(100 - \%\text{ moisture} - \%\text{ fat})}{100}$$

4.2.6 Spectral acquisition

Sardine samples were scanned using an ultra-compact hand-held Viavi's MicroNIR 1700 spectrophotometer (Viavi Solutions Inc., Milpitas, CA, USA), achieving a pixel size (resolution) of 30 µm x 250 µm/50 µm. The external white reference used was a diffuse reflectance standard (Viavi Solutions Inc., Milpitas, CA, USA). The 100% absorbance measurements were performed every 10 min during the sample acquisition period. Scanning took place in reflectance mode at 6.2 nm intervals within the wavelength range of 908 to 1650 nm. Each scan was the average of

64 individual scans (scanning parameter). MicroNIR software (Viavi Solutions Inc., Milpitas, CA, USA) was used to perform the spectroscopic measurements. Only the homogenised fish samples were used for NIR spectroscopy analysis. These samples were placed individually in a custom-made hollowed-out Teflon (PFTE) disk (Maizey's (Pty) Ltd, Kuilsriver, South Africa) that completely covered the contactable collar of the MicroNIR spectrophotometer upon scanning. For each sample, duplicate scans were taken, and the Teflon disc was cleaned after consecutive samples. All spectral data were saved in Microsoft Excel format for further analysis in The Unscrambler software (Camo ASA, Oslo, Norway) version 10.4.

4.2.7 Multivariate data analysis

4.2.7.1 *Outlier detection*

Principal component analysis (PCA) was used to examine possible grouping of samples and to identify possible spectral outliers before using the data set to develop PLS models. Before any data interpretation, outlier samples from the population, due to errors in the scanning procedure and high variability between the samples, were consequently omitted. PCA influence score plots and Hotelling's T^2 plots were used to identify the outlying samples.

4.2.7.2 *Spectral pre-treatment*

Because NIR spectra are affected by particle size, light, and path-length variations, pre-treatment of the spectral data improves calibration accuracy. Therefore, spectral data pre-treatments such as multiplicative scatter correction (MSC) and first or second order derivatives, as well as Savitzky-Golay (S-G) smoothing, were applied to the spectra to reduce the noise and light scattering effects. Different pre-treatment options were explored, and the best data pre-treatment was selected based on the minimum standard error of cross-validation (SECV). Furthermore, for the calibration of the moisture content, the wavelength range from 908 to 1199 nm was excluded due to high signal to noise ratio. For protein, fat and ash content predictions, there were no wavelengths deleted, and the entire range from 908 to 1680 nm was used.

4.2.7.3 Descriptive statistics

Descriptive statistics were used to view some essential characteristics of the data, i.e. mean, range (minimum and maximum values) and standard deviation (SD).

4.2.7.4 Partial least squares (PLS) regression

The appropriate chemometric techniques for regression purposes were performed in The Unscrambler V.10.4 (Camo, Oslo, Norway) statistical software program. Separate datasets were constructed for each proximate constituent. Each of these sample sets were separated into calibration and validation sets (with roughly three times more samples present in the calibration sets than in the prediction sets). The calibration sets were used to develop the calibration models. The developed models were validated by using samples included in the validation set which had not been previously used in the calibration procedure.

The number of factors used as variables in the prediction equations was fixed at a maximum of ten. Full cross-validation was applied to select the optimum number of factors. External validation was used to test the calibration model and the accuracy of the respective predictions were evaluated in terms of the root mean square error of calibration (RMSEC), the root mean square error of prediction (validation) (RMSEP) and the coefficient of determination of both the calibration (R^2_c) and the validation (R^2_v). The ratio between the standard deviation (SD) of the data set (population) of the validation set and the standard error of prediction (RMSEP) was also calculated and is known as the Ratio Performance Deviation (RPD) (Saeed *et al*., 2012). The RPD shows how good the calibration and prediction will work for analytical purposes. An SD/SEP value higher than 3.0 is considered very rough for screening purposes, and a value higher than five is considered acceptable for quality control (Saeed *et al*., 2012).

The Unscrambler v.10.4 (Camo, Oslo, Norway) software was used to perform data treatment such as MSC and derivatives, also for the PLS modelling of the chemical data of the fish samples.

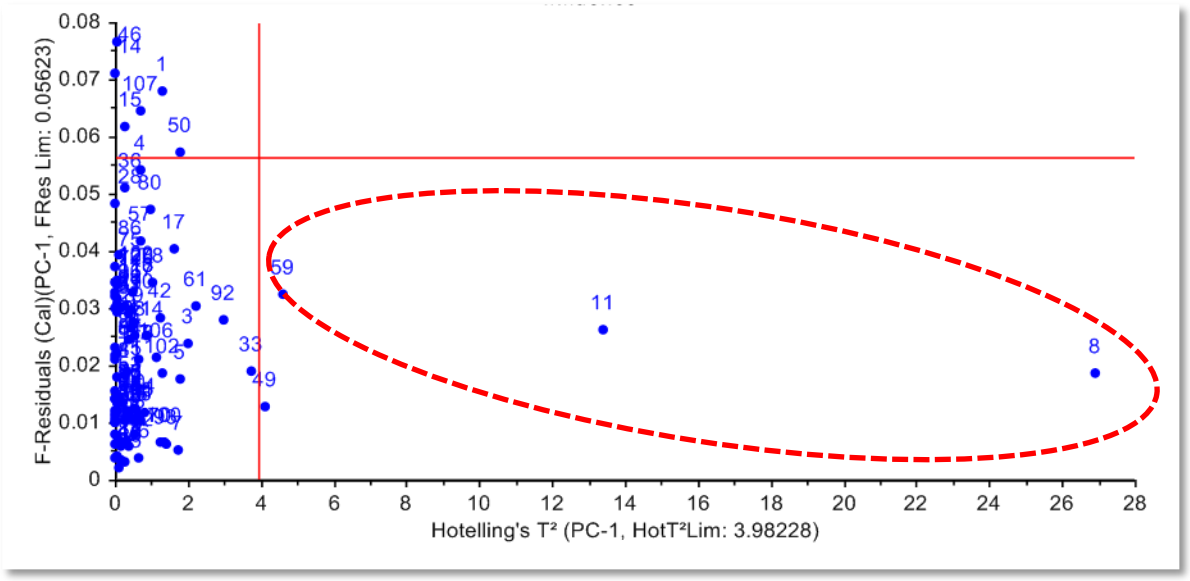
4.3 Results and discussion

4.3.1 Outlier detection

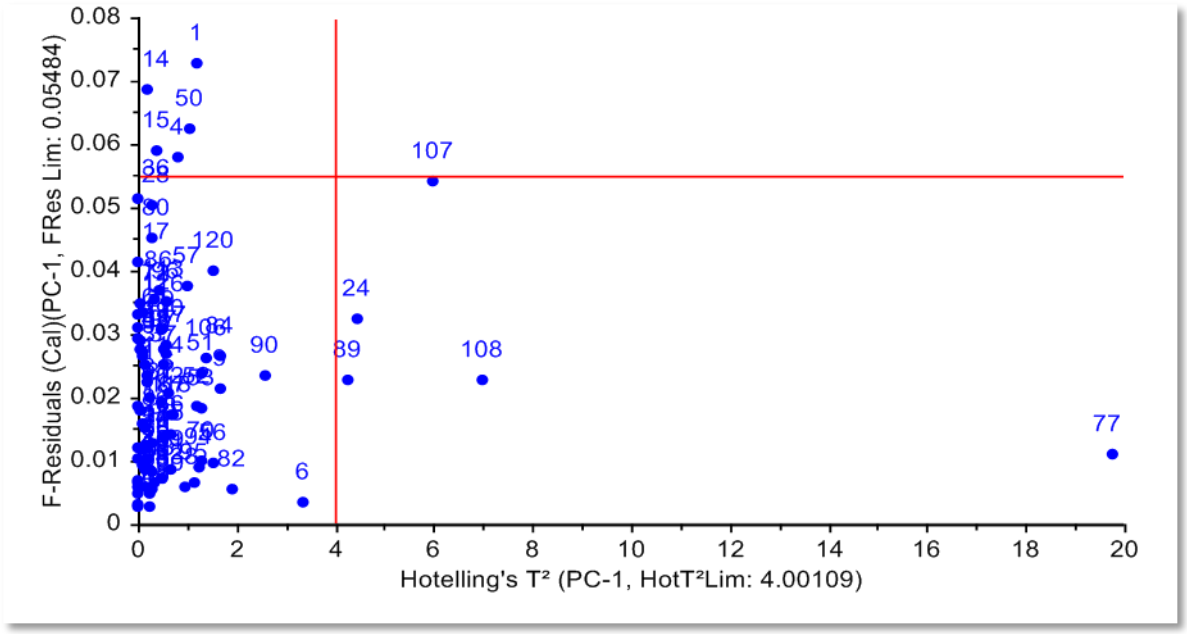
PCA was applied on spectral data to identify possible outliers in the data set. The primary tool for outlier detection was the Influence plot, which was presented when performing a PCA, with Hotelling's T^2 as the x-axis and PCA residual as the y-axis. As depicted on the plot, samples that are found in the bottom right quadrant are seen as outliers. From the Influence plot of the moisture content data set (Fig. 4.1 (a)) samples, number 8 and 11 were identified as being outliers and consequently removed. For the ash content data set, the PCA Influence plot was without outliers, and no samples were deleted. The fat content data set showed one extreme outlier due to that sample number 77 being situated far from the rest of the samples in the PCA Influence plot (Fig.

4.1 (b)). This sample was removed from the dataset. Likewise, for the protein content data set, one sample, i.e. sample number 34 was a definite outlier (Fig. 4.1 (c)) and therefore removed from the respective data set.

(a)



(b)



(c)

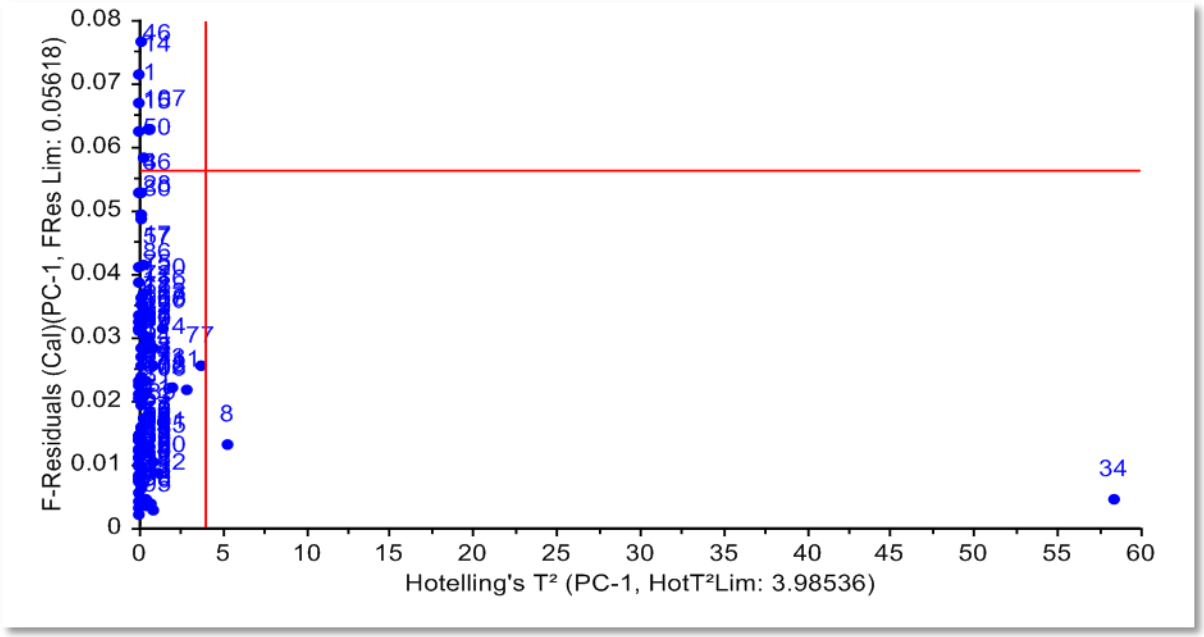


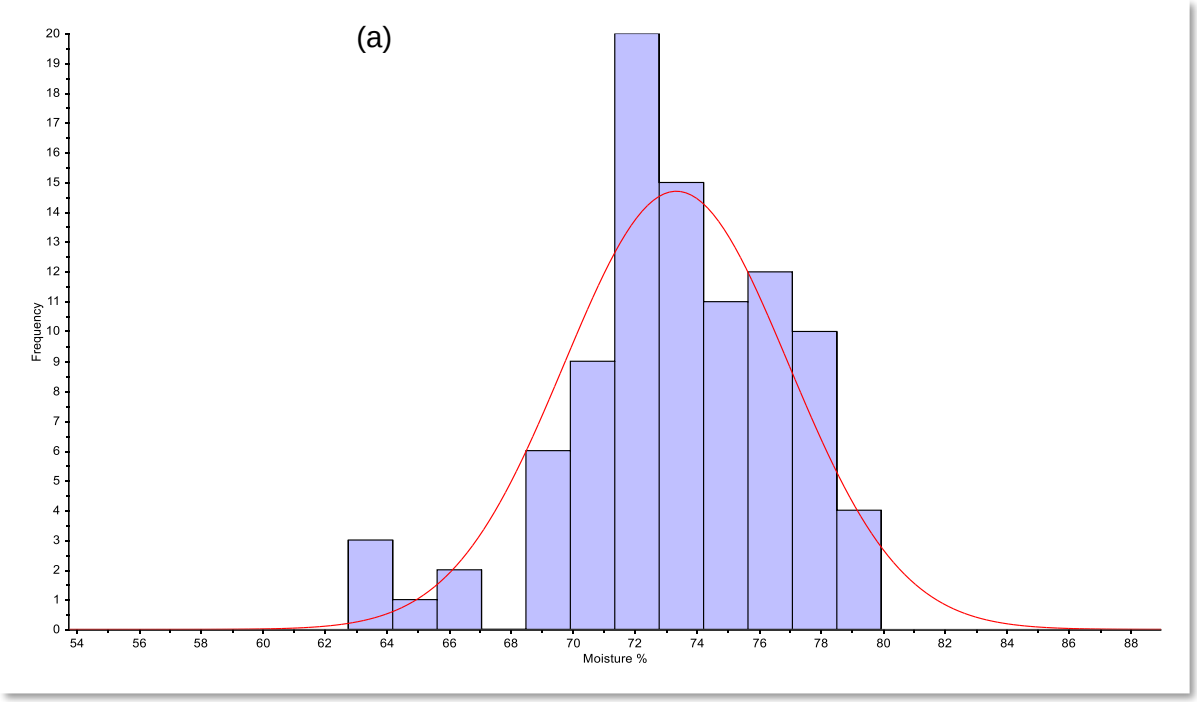
Figure 4. 1: PCA Influence plots for the respective datasets: a) moisture content, (b) fat content and (c) protein content with the respective outliers.

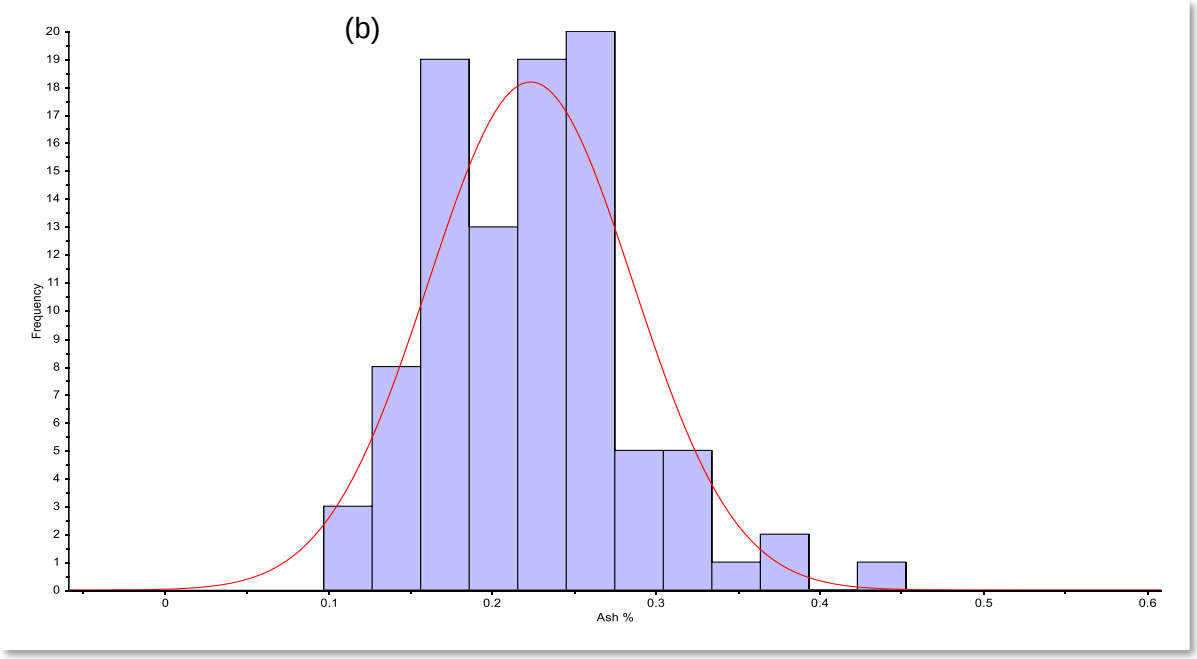
4.3.2 Descriptive statistics

Descriptive statistics were used to view some basic characteristics of the respective data sets with regards to minimum values, maximum values, the means and the standard deviation (SD) associated with each constituent as determined using the conventional analytical methods (Table 4.1). In a former study by Bulla *et al*. (2011) also on sardines (*Sardinella brasiliensis*), a similar proximate composition was found when conventional methods were used. These researchers reported mean values of 73.48% for moisture, 0.23% for ash, 5.47% for fat (lipid) and 19.53% for protein. Additionally, histograms (Figs. 4.2 (a) to (d)) are presented as an overview of the distribution of proximate values for each respective data set. There was a vast range found in the lipid % dataset (0.28% to 16.86%). Therefore, samples with extreme low lipid were excluded from the respective datasets. Also, the obvious outliers identified by the PCA influence plots were removed. The datasets used for the PLS regression models consisted of the following number of samples (Table 4.1): moisture % = 93, ash % = 96, lipid % = 84 and protein % = 93.

Table 4.1: Overview of the proximate composition of the respective sardine samples

	Number of samples (n)	**Mean (%)**	**Minimum (%)**	**Maximum (%)**	**Standard deviation (%)**
Moisture %	93	73.35	62.76	79.95	3.6
Ash %	96	0.22	0.09	0.45	0.06
Lipid %	84	5.75	0.28	16.86	3.63
Protein %	93	19.04	1.6	28. 82	3.67


(a)
Frequency
Moisture %
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
54
56
58
60
62
64
66
68
70
72
74
76
78
80
82
84
86
88


(b)
Frequency
Ash %
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
0
0.1
0.2
0.3
0.4
0.5
0.6

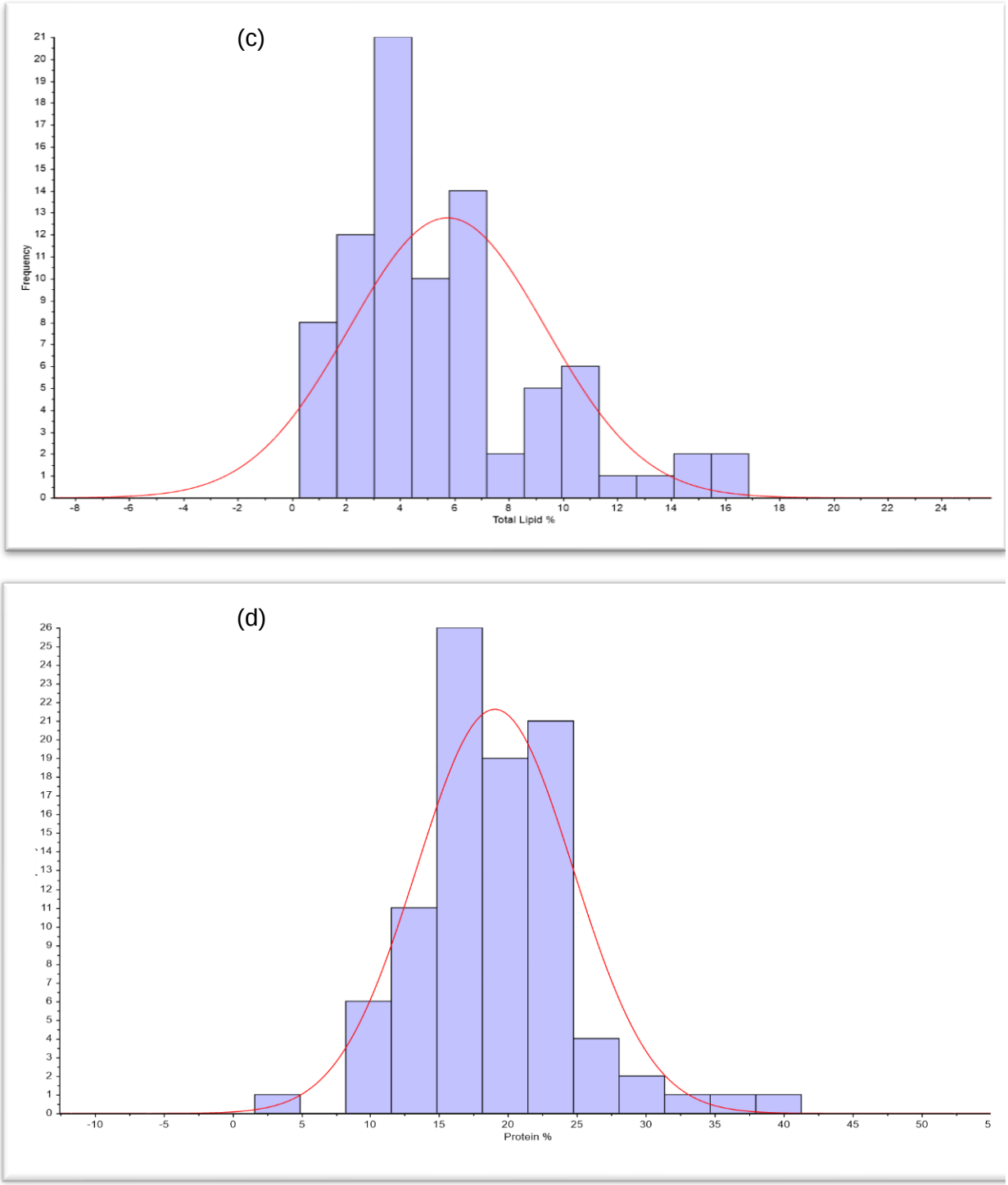


Figure 4. 2: Histograms indicating the distribution (or range) of the respective constituents for each data set: (a) moisture content, (b) ash content, (c) lipid content and (d) protein content after the removal of outliers

4.3.3 Partial least square regression

The respective sample sets were separated into calibration sets as well as validation sets as summarised in Table 4.2. The calibration sets were used to develop the calibration models after which the developed models were evaluated by using samples included in the validation sets which had not been previously used in the calibration procedure. The pre-processing options that resulted in the lowest prediction errors were MSC, second derivative S-G with second polynomial order and three smoothing points or a combination thereof. This was applied to all the spectra, regardless of being calibration or validation samples.

Table 4.2: Descriptive statistics of the calibration, as well as validation data sets for moisture %, ash%, lipid % and protein % contents of SA sardine samples

	Calibration set				Validation set			
	N	**mean**	**Min**	**Max**	**N**	**Mean**	**Min**	**Max**
Moisture (%)	69	73.13	62.76	79.63	24	73.95	63.76	79.95
Ash (%)	72	0.22	0.10	0.45	24	0.21	0.09	0.31
Lipid (%)	58	6.24	2.26	16.86	16	6.71	2.58	14.65
Protein (%)	69	19.71	8.53	28.82	24	17.13	10.98	24.51

n: number of samples; min: minimum; max: maximum

The NIR spectroscopy calibration and validation statistics for the moisture, ash, lipid % and protein % are shown in Table 4.3, along with the preferred pre-processing that was applied to the respective spectra. For the moisture % PLS regression calibration, the best number of factors to be used as indicated by the Explained variance plot (Fig. 4.3 a) was 5. The choice of 5 factors came from the red validation line that indicated an over-optimistic PLS-model when using more than five factors (the line crossed over to the negative side of the plot) (Fig. 4.3 a). From the Explained variance plot, a lack of structure in the data was depicted as the first couple of factors struggled to explain enough variation. As indicated by the plot, to describe 50% of the variation in the data, five factors were needed (blue line). This pointed to much noise in the data set, or in other words, unexplained variance (Esbensen, 1994).

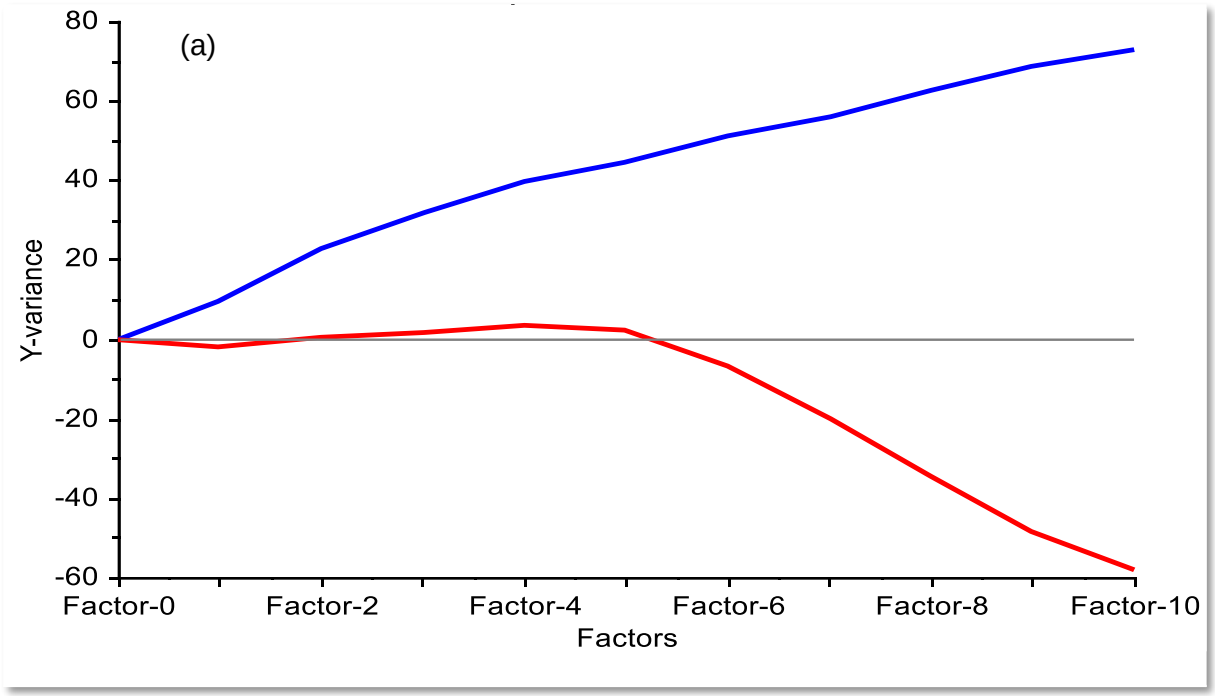
From the Predicted vs. Reference plot (Fig. 4.3 (b)), when using 5 factors, it could be observed that the coefficient of determination of calibration (R^2_c) and the standard error of calibration (SEC) for the estimation of the moisture % in homogenised SA sardine samples was not very good (R^2_c = 0.44, SEC = 2.65%) (Table 4.3). External validation was used to test the

calibration model, and the accuracy of prediction was evaluated in terms of the coefficient of determination of the external validation (R^2_v) and standard error of prediction (SEP), which was 0.22 and 3.13%, respectively (Table 4.3). To test the applicability of this calibration in terms of the RPD value (SD/SEP), a low value of only 1.15 was obtained, indicating a calibration that is not good enough to replace the conventional method but could be used for rough screening (Table 4.3).

Table 4.3: Prediction of the proximate composition corresponding to the homogenised SA sardine samples

	Calibration				Validation		
	Pre-processing	**Number of factors**	**RMSECV (%)**	**R^2_c**	**RMSEP (%)**	**R^2_v**	**RPD**
Moisture %	S-G 2nd Der 2nd pol. 3 s.p.	5	2.65	0.44	3.14	0.22	1.15
Ash %	MSC	8	0.055	0.27	.056	0.24	1.07
Lipid %	S-G 2nd Der 2nd pol. 3 s.p.	7	0.177	0.70	2.46	0.61	1.47
Protein %	MSC S-G 2nd Der 2nd pol. 3 s.p.	7	4.17	0.46	3.41	0.22	1.07

RMSEC: Root mean square error of calibration; R^2_c: coefficient of determination of the calibration; RMSEP: root mean square error of the prediction; R^2_v: coefficient of determination of the validation; RPD: Ratio Performance Deviation; mean centering: m-c; Savitzky-Golay second derivative with second polynomial order and 3 points smoothing; MSC: multiplicative scatter correction.



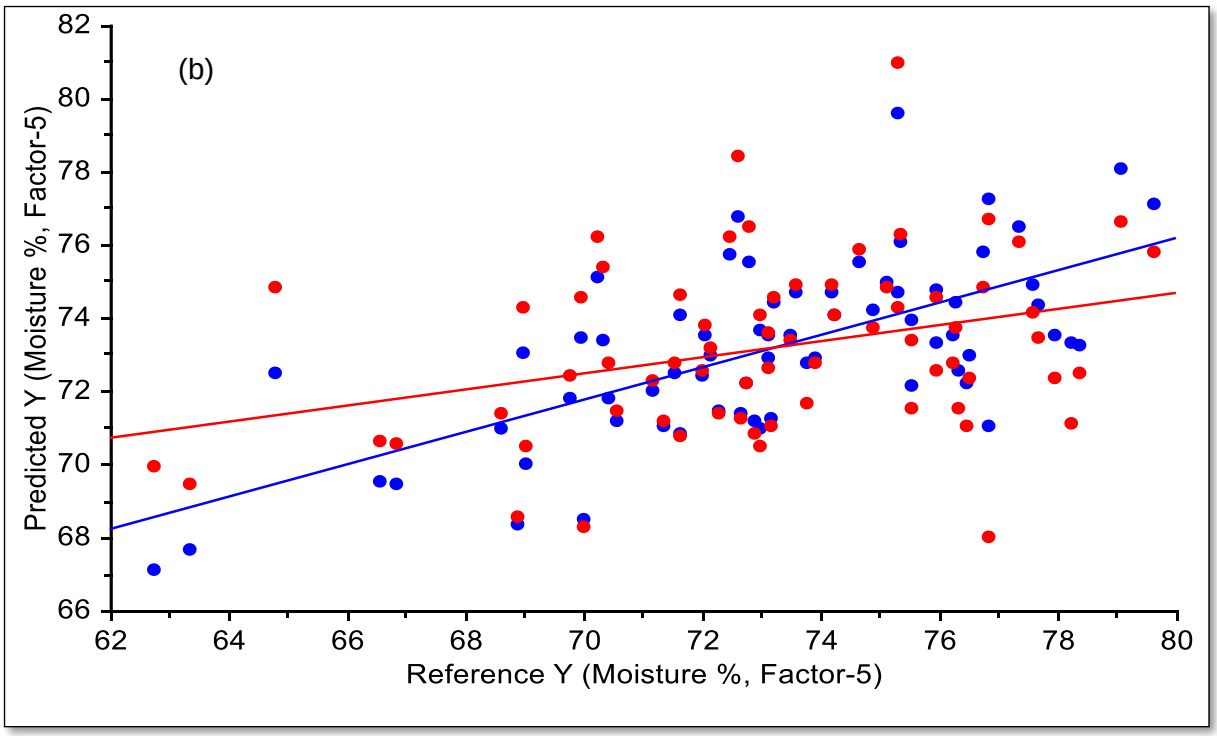


Figure 4.3: NIR spectroscopy calibration and validation plots for moisture %: (a) Explained variance and (b) Predicted vs. Reference plot.

In the same manner, when a PLS regression model was performed on the ash % data set, the optimum number of PLS factors were 8, as indicated by the relevant Explained variance plot. In this instance, the pre-processing that resulted in the lowest prediction error was MSC in combination with mean centering (Table 4.3). As expected from the model that struggled to explain enough variance, the Predicted vs. Reference plot (Fig. 4.4) did not show a good regression coefficient (R^2_c = 0.27), and the error associated with the prediction was 0.055% ash (Table 4.3). For the external validation set, the R^2_v = 0.24 with a SEP of 0.056% (Table 4.3). When the RPD value was calculated using the SEP divided by the SD associated with the ash% determination, a result of 1.07 was obtained, which was also only good enough to be used as a very rough screening method.

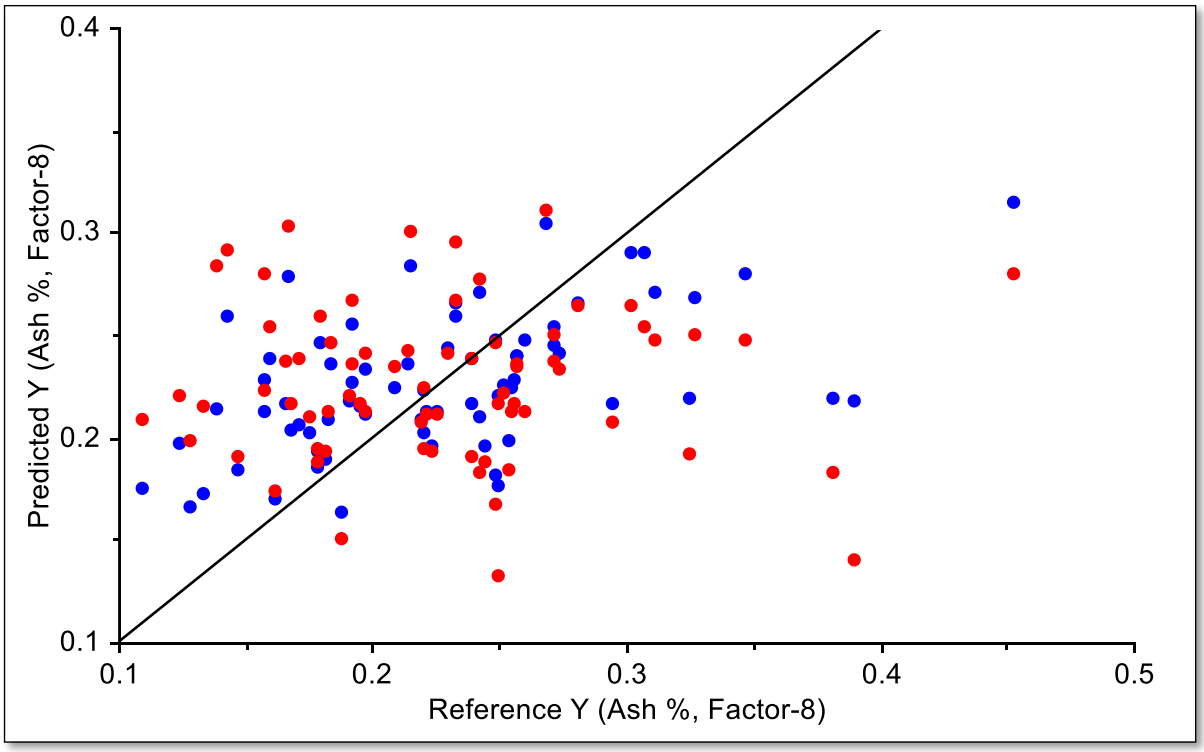


Figure 4.4: A Predicted vs. Reference plot of the PLS regression model using the ash content (%) data set.

The NIR spectroscopy PLS regression model performed on the fat (or lipid) percentage dataset resulted in a promising prediction calibration model. As can be seen from the Predicted vs Reference plot (Fig. 4.5) a fair regression coefficient was obtained (R^2_c = 0.70) for the calibration

set with a SEC of 1.77% (Table 4.3). This was when the pre-processing combination of mean-centering, second derivative S-G with second polynomial order and three smoothing points were used. For the validation set, the R^2_v = 0.61 with an error of prediction (SEP) of 2.46% (Table 4.3). To put these values into perspective (or to judge the usefulness of the calibration), the RPD value was determined (SD/SEP = 3.63% / 2.46%), and a value of 1.47 was obtained (Table 4.3). The prediction model was also not useful for screening purposes, despite the promising regression coefficient from the calibration set. The lack of structure in the data, or in other words, the excessive noise included in the spectral data prevented the prediction models to be remotely optimal.

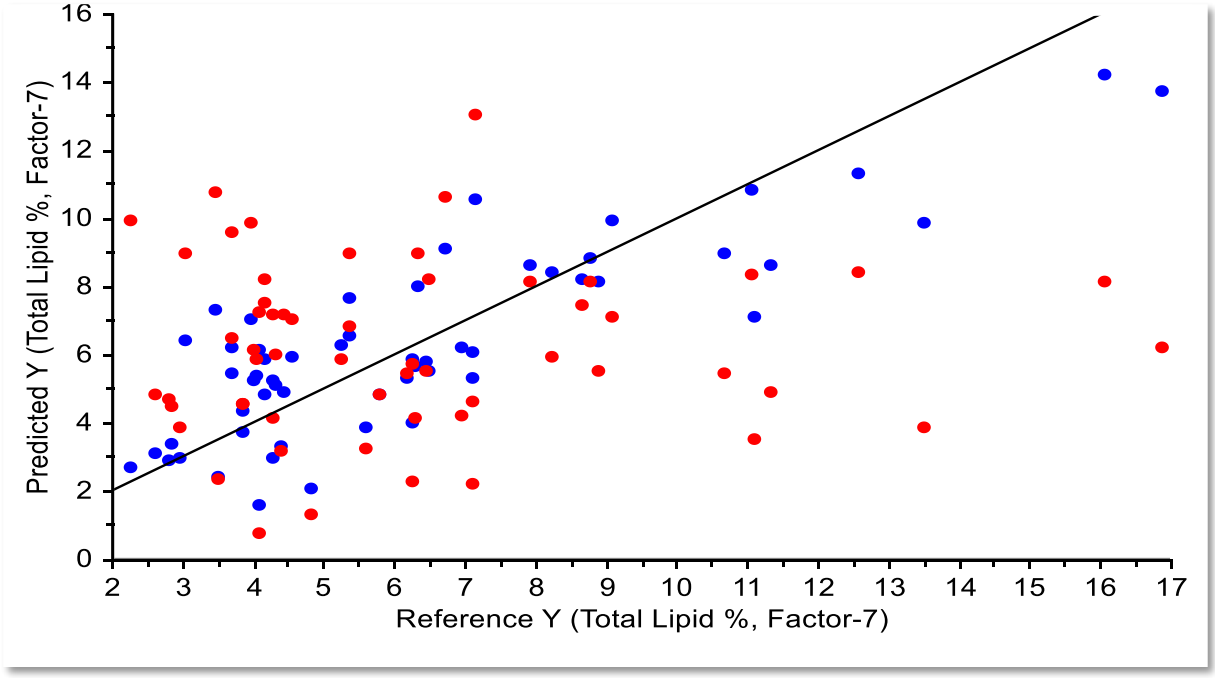


Figure 4. 5: A Predicted vs. Reference plot of the PLS regression model using the fat % data set.

Lastly, for the PLS regression model using protein % data, the pre-processing combination was that of S-G second derivative as well as MSC. Seven PLS factors were used (as indicated by a full-cross validation step), and the SEC = 4.17%, whereas the R^2_c = 0.46. For the validation step the SEP = 3.41 and the R^2_v = 0.22. An RPD value of 1.07 was obtained as the SD of the set = 3.67% for the protein determinations when using the conventional method. Again, it would not be

advised to replace the conventional method with this NIR spectroscopy prediction method, as the error associated with these predictions was not low enough.

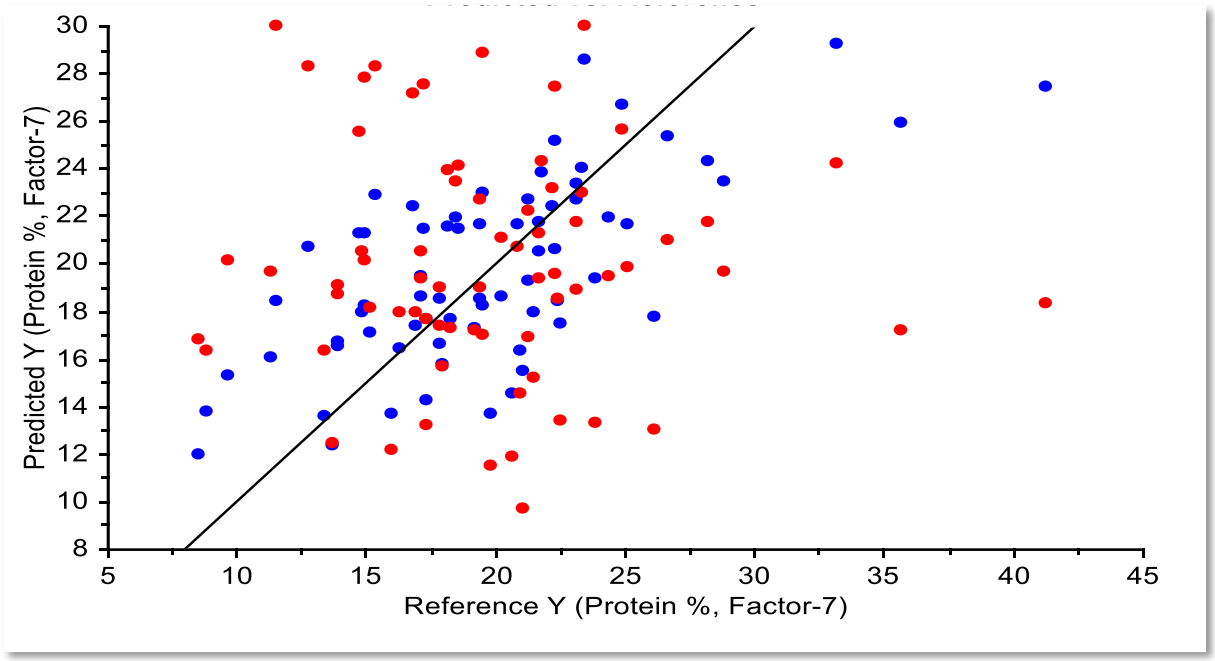


Figure 4.6: A Predicted vs. Reference plot of the PLS regression model using the protein % data set.

In comparison with the above tabulated results (Table 4.3), Khodabux *et al*. (2007); Saeed *et al*. (2012) and Xiccato *et al*. (2014) provided reasonable coefficients for predictions of moisture (R^2> 0.70), protein, (R^2 >0.60) and fat (R^2 > 0.70) when analysing tuna fishes (*Katsuwonus pelamis* and *Thunnus albacares*), sea bass (*Dicentrarchus labrax L*.) and herring (*Clupea harengus*) and sardine (*Sardinops sagax*). The poor prediction results obtained from the SA sardine study compared to other studies were most likely due to the wide chemical variations of the species such as sex. Different fishing locations and maturity stage also contributed to poor predictions. The variation in the study was also due to prolonged freezing period of sardine samples prior to NIR spectral acquisition (Nortvedt *et al*., 1998). NIR spectroscopy prediction of protein in fish may be compromised by the presence of several non-protein nitrogen compounds (Rasco *et al*. 1991). A variation in sample temperature could also be a possible source of error in NIR calibration (Williams *et al*., 1982).

4.4 Conclusion

This study demonstrated that proximate constituents such as lipid (%) could not be predicted using PLS prediction models based on NIR data obtained using the portable MicroNIR spectrophotometer. The other proximate constituents, i.e. moisture, ash and protein (%) also could not be predicted accurately using PLS prediction models as developed on this study. The prediction models could be improved by extending the number of samples in the sample sets and by using reference methods with smaller SD amongst measurements. The predictive performance demonstrated that NIR spectroscopy could not to be used as a rough screening tool for quality parameter measurements for the number of samples that were analysed for this thesis data set.

References

Adewumi, A.A., Adewole, H.A. & Olaleye, V.F. (2014). The proximate and elemental composition of the fillets of some fish species in Osinmo Reservoir, Nigeria. Agriculture and Biology journal of North America, **5**(3), 109-117.

AOAC International (2002) Official methods of analysis (17th edition), Arlington, Virginia, USA: Association of Official Analytical Chemists Inc. Protein (Crude) in Animal Feed and Pet Food. AOAC Official Method 992.15.

AOAC International (2012) Official methods of analysis (17th edition), Arlington, Virginia, USA: Association of Official Analytical Chemists Inc. Loss on drying (moisture) at 95 – 100°C for feeds. AOAC Official Method 934.01.

AOAC International (2002). Official methods of analysis (17th edition), Ash of Animal Feed. AOAC Official Method 942.05.

Azim, M.A., Islam, M.R., Hossain, M.B. & Minar, M.H. (2012). Seasonal variations in proximate composition of gagentic Sillago, Sillagnopsis panyus (*Perciformes sillagnidae*). *Middle-East Journal of Scientific Research,* **11**(5), 559-562.

Bagthasingh, C., Aran, S.S., Vetri, V. & Innocen, A. (2016). Seasonal variation in the proximate composition of sardine (*Sardinella gibbosa*) from Thoothukudi coast*. Indian Journal of Geo-Marine Science*, **45**, 800–806.

Baeten, V. & Dardenne, P. (2002). Spectroscopy: Developments in instrumentation and analysis. *Grasas y Aceites*, **53**, 45-63.

Begum, M. & Minar, M.H. (2012). Comparative study about body composition of different SIS, shell fish and ILISH commonly available in Bangladesh. *Trends in Fisheries Research*, **1**(1), 38-42.

Briggs, G.M. & Schweigert, B. (1990). An overview of meat in the diet. *Advances in Meat Research*, **6**. Pp.1–18. New York, USA: Elsevier Applied Science.

Bulla, M.K., Simionato, J.I., Matsushita, M., Garcia Coro, F.A., Shimokomaki, M., Visentainer, J.V. & de Souza, N.E. (2011). Proximate composition and fatty acid profile of raw and roasted salt-dried sardines (*Sardinella brasiliensis*). *Food and Nutrition Sciences*, **2**, 440-443.

Capanio, F., Lestingi, A., Summo, C., Bilancia, M.T. & Laudadio, V. (2004). Chemical characteristics and lipid fraction quality of sardines (*Sardina pilchardus W*.): influence of sex and length. *Journal of Applied Ichthyology*, **20**, 530-535.

Dahlman, N. (2012). Near-infrared spectroscopy- An introductory study on measurement techniques and tools for analysing content in pulp and paper media. Masters Thesis. Karlstads University, Sweden.

Downey, G. (1996). Non-invasive and non-destructive percutaneous analysis of farmed salmon flesh by near infrared spectroscopy. *Food Chemistry*, **55**(3), 305-311.

Esbensen, H.H. (2002). Principal component analysis (PCA). In: *Multivariate Data Analysis in practice,* 5th Edition, Pp. 27 – 34.

Garcia-Jares, C.M. & Medina, B. (1997). Application of multivariate calibration to the simultaneous routine determination of ethanol, glycerol, fructose, glucose, and total residual sugars in botrytized-grape sweet wines by means of near infrared reflectance spectroscopy. *Fresenius Journal of Analytical Chemistry*, **357**, 86–91.

Geiger, E. & Borgstrom, G. (1962). Fish Protein - Nutritive Aspects. In: Fish as Food (edited by G. Borgstrom). Pp. 29-114. New York: Academic Press Inc.

Greenfield, H. & Southgate, A. (2003). Food composition data. *Food and Agriculture Organization of the United Nation*, 2nd edition. Rome.

Huss, H.H. (1988). Chemical composition. In: *Fresh fish: quality and quality changes*, Pp. 15-25. Rome: Food and Agriculture Organization of the United Nations Danish International Development Agency.

Issakson, T. Togersen, G., Iversen, A. & Hildrum, K.I. (1995). Non-destructive determination of fat, moisture and protein in salmon fillets by use of near-infrared spectroscopy. *Journal of the Science of Food and Agriculture*, **69**(1), 95-100.

Jacquot, R. (1961). Organic Constituents of Fish and Other Aquatic Animal Foods. In: *Fish as Food. Production, Biochemistry and Microbiology* (edited by G. Borgstrom). Pp. 145-209. New York, London: Academic Press.

Jhaumeer-Laullo, S., Ramasami, P. (2005). Infrared spectroscopy; An analytical tool in Food Science, Proceedings of 7th MAS, Mauritius.

Khitouni, I.K., Boudhrioua, M., Bouain, A. & Rebah, F.B. (2014). Seasonal variations in proximate and fatty acid composition of golden grey mullet Liza aurata (R, 1810) from Tunisian coast. *International Journal of Agricultural Policy and Research*, **2**(7), 273-280.

Lee, C.M., Trevino, B., Chaiyawat, M. (1996). A simple and rapid solvent extraction method for determining total lipids in fish tissue. *Journal of AOAC International*, **79,** 487-492.

Modibbo, U.U., Osemeahon, S.A., Shagal, M.H. & Halilu, M. (2014). Effect of moisture content on the drying rate using traditional open sun and shade drying of fish from Njuwa Lake in North Eastern Nigeria. *IOSR Journal of Applied Chemistry*, **7**(1), 41-45.

Mota da Silva, V., Silva e jailson, L.A. & De Andrade, J.B. (2008). Determination of moisture content and water activity in algae and fish by thermoanalytical techniques. *Química Nova*, **31**(4), 901-905.

Nortvedt, R., Torrissen, O.J. & Tuene, S. (1998). Application of near-infrared spectroscopy in the determination of fat, protein and dry matter in Atlantic halibut fillet. *Chemometrics and Intelligent Laboratory Systems*, **42**, 199-207.

Osborne, B.G., Fearn, T. & Hindle, P.H. (1993). Practical NIR Spectroscopy with applications in food and beverage analysis, 2nd edition, Pp. 1-220. England: Longman Group UK Limited.

Pirestani, S., Ali Sahari, M., Barzegar, M & Seyfabadi, S.J. (2009). Chemical compositions and mineral of some commercially important fish species from the South Caspian Sea. *International Food Research Journal*, **16**, 39-44.

Rasco, B.A., Miller, C.E. & King, T.L. (1991). Utilization of NIR spectroscopy to estimate the proximate composition of trout muscle with minimal sample pre-treatment. *Journal of Agricultural Food Chemistry*, **39**, 67-72.

Simhachalam, G., Sampath Kumar, N.S. & Rao, K.G. (2015). Biochemical composition and nutritional value of *Streptocephalus simplex* as live feed in ornamental fish culture. *The Journal of Basic Applied Zoology*, **72**, 66-72.

Tacon, A.G.J. & Metian, M. (2013). Fish matters: importance of marine foods in human nutrition and global supply. *Reviews in Fisheries Sciences*, **21**(1), 22-38.

Williams, P.C., Norris, K.H. & Zarowski, W.S. (1982). Influence of temperature on estimation of protein and moisture in wheat. *Cereal Chemistry*, **59**, 473-477.

Xiccato, G., Trocino, A., Tulli, F. & Tibaldi, E. (2004). Prediction of chemical composition and origin identification of European sea bass (*Dicentrarchus labrax* L) by near infrared reflectance spectroscopy (NIRS). *Food Chemistry*, **86**, 275-281.

YÁÑEZ, E., Ballester, D., Monckeberg, F., Heimlich, W. & Rutman, M. (1976). Enzymatic fish protein hydrolyzate: chemical composition, nutritive value and use as a supplement to cereal protein. *Journal of Food Science*, **41**, 1289-1292.

5 CHAPTER FIVE

PREDICTING THE FATTY ACID COMPOSITION OF SOUTH AFRICAN SARDINES, USING NEAR INFRARED (NIR) SPECTROSCOPY AND PARTIAL LEAST SQUARES (PLS) REGRESSION MODELLING

Abstract

Various marine fish species are known to be excellent sources of fatty acids, especially the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The fatty acid composition of SA sardines (*Sardinops sagax ocellatus*) was determined to quantify the individual fatty acids using gas chromatography. As an alternative to gas chromatography, NIR spectroscopy was used to obtain NIR scans, and with PLS prediction models, predict the fatty acid composition of the respective sardine samples. NIR spectra of whole sardine samples were acquired using a handheld MicroNIR instrument covering the wavelength range 908 to 1680 nm. The fatty acid composition as obtained by gas chromatography was used as reference values for the construction of partial least squares (PLS) regression models. NIR spectra were corrected with treatments such as multiplicative scatter correction (MSC) and second derivative Savitzky-Golay smoothing. The developed PLS models provided a coefficient of determination for the validation model (R^2_v) of 0.48, a SEP of 0.45% and a RPD value of 0.65 for saturated fatty acids (SFA), whereas R^2_v = 0.48, SEP = 0.44% and RPD value was 1.47 for the polyunsaturated fatty acids (PUFA). Prediction results for monounsaturated fatty acids (MUFA) were poor with very little correlation found between the predicted and reference values (R^2_c = 0.38, SEC = 2.33). Similarly,

PLS models for total fatty acids (TFA) also resulted in poor predictions (R^2_c = 0.37, SEC = 2.03). Thus, it was evident that NIR spectroscopy did not predict these constituents to a satisfactory level.

5.1 Introduction

SA sardine (*Sardinops sagax ocellatus*), which is an important forage species abundantly found in the Benguela ecosystem, is a commercially crucial fatty fish in both domestic and foreign markets (Van der Lingen 2002; Çelik e*t al*., 2005; Coetzee *et al*., 2008). In addition to the high biological value of its proteins, sardines consist of a significant amount of polyunsaturated fatty acids (PUFA) such as omega-3 fatty acids, which contributes to its valuable nutritional and functional characteristics (Vasconcellos, 2003 & Tarley *et al*.,2004; Xu *et al*., 2015; Bagthasingh *et al*., 2016). Nowadays, it is known that fish fatty acids are the most beneficial type of fatty acids concerning human health (Çelik e*t al*., 2005*;* Rodrigues *et al*., 2017; Ural *et al*., 2017).

The lipid fraction of fish is the subject of a great deal of attention due to its high content of n-3 PUFA [eicosapentaenoic acid, 20:5n-3 (EPA) and docosahexaenoic acid, 22:6n-3 (DHA)], which have demonstrated a positive effect in the prevention of specific human diseases (Haliloglu *et al*., 2004; Gonzalez *et al*., 2006; Ural *et al*., 2017). It has been found that the lipid content of European sardine (*Sardina pilchardus W*) has an extensive range (0.5% to 20%) depending on the season, stage of sexual maturity and body size (Hardy & Keay, 1972; Christie, 1987; Nunes *et al*., 1991; Bandarra *et al*., 1997). For the Indian species *Sardinella longiceps*, a lipid content found in their muscle is about 3% to 4% in June-July and increases up to 18% in November-December. For European sardines (*Sardinella pilchardus*) the lipid content in the muscle varies between 1.2% and 18%. In Japanese sardines (*S. melanostidus*) the lipid content was found to be very low in February (1.8%) and high in July and September (7.2%) (Bagthasingh *et al*., 2016).

Fish lipids are characterised by a large group of saturated and unsaturated (monounsaturated and polyunsaturated) fatty acids which are associated with mixed triglycerides (Bystrickà & Ďuračkovà, 2016). Saturated fatty acids (SFA) belong to non-essential fatty acids and may be harmful if consumed in excess (Hussein, 2013). Monounsaturated (MUFA) is primarily characterised by omega-9 fatty acids and have beneficial health effects on the cholesterol metabolism, protective role against cardiovascular diseases and furthermore are less sensitive to oxidative damage (Hussein, 2013; Zamaria, 2004).

Sardine lipids, in general, are rich in long-chain n-3 PUFAs, namely EPA and DHA, which are generally known to play an essential role in human health (Çelik e*t al*., 2005; Bulla *et al*., 2011; Xu *et al*., 2015; Bagthasingh *et al*., 2016). EPA and DHA are generally known to play an essential role in human health (Çelik e*t al*., 2005; Bulla *et al*., 2011; Xu *et al*., 2015; Bagthasingh *et al*., 2016). Both EPA and DHA proved to decrease the risk of diseases such cardiovascular, coronary heart and circulatory diseases in humans (Eder, 1995; Schmitt-Rozieres *et al*., 2000; Çelik e*t al*., 2005; Khitouni *et al*., 2014; Xu *et al*., 2015). EPA and DHA also play an essential role in the development and functioning of the brain and retina (Bulla *et al*., 2011).

PUFA composition varies among different fish species of both freshwater and marine origins (Jabeen & Chaudhry, 2011). For fish to adapt to the low water temperatures, it requires PUFA (Chanmugam *et al*., 1986). Cold and deep sea fish have fatty acids in abundance, and the melting temperatures of n-3 fatty acids are lower than n-6 fatty acids. It is therefore estimated that n-3 PUFA contents of fish in warm water are lower (Chanmugam *et al*., 1986; Rahman *et al*., 1995).

In a study conducted by Zlatanos and laskaridis, (2007) in Mediterranean sardine (*Sardina pilchardus*), SFA were found to increase during months of high fat content (Zlatanos & Lskaridis, 2007). Luzia *et al*., 2003 reported high fatty acid contents in sardines analysed in summer. Therefore, SFA concentrations increase during periods of enhanced feeding activity (Shirai *et al*., 2002; Gockse *et al*., 2004). The variances mentioned above can generally be attributed to the fact that fish consists of two basic muscle types, i.e. red muscle tissue and white muscle tissue. The red muscle is used for slow, continuous movements, whereas the white muscle is used for rapid, sudden movements (Love, 1980). Thus, fish species that display a high level of activity will have a more significant amount of white muscle tissue than those that are relatively inactive (Love, 1988).

Additionally, the fatty acid profile of fish muscle is influenced by several environmental and internal factors such as the diet, starvation, genetic background, age, sex and maturation (Çelik e*t al*., 2005; Mraz & Pickova, 2011; Yeşilayer & GenÇ, 2013). The plankton of marine feeds presents low levels of n-6 PUFA of which EPA is the predominant fatty acid. However, few reports of the relationship between the fatty acid composition of sardine oil and plankton have been presented (Shirai *et al*., 2002). Variations in the fatty acid profile may also be influenced by the spawning cycle and the gonadosomatic index in the sardine *Sardinops sagax* (Gámez-Meza, 1999; Zlatanos & laskaridis, 2007).

Table 5.1: An overview of three commonly used methods for fatty acid determinations, including the respective principles, advantages, as well as disadvantages

Method	Principle	Advantages	Disadvantages	Reference
Thin layer chromatography (TLC)	Separation of organic compounds is achieved on a stationary phase due to differences in polarity of the analytes	TLC is inexpensive. Variations of the mobile phase allow the separation of the complex mixtures.	Unsaturated lipids may be oxidised if the TLC plate is stored for a while since large lipid surface is exposed to the atmosphere	Fuchs *et al*., 2011
High-performance liquid chromatography (HPLC)	Separation of all organic chemicals (independent of polarity or volatility) on a stationary phase under high pressure by elution with different solvents	High-quality separations are achieved.	It is difficult to detect saturated lipids due to the lack of Ultra Violet (UV) absorptions. HPLC is more expensive and time-consuming	Lehotay & Hajšlová, 2002; Fuchs *et al*., 2011
Gas chromatography (GC)	Chromatographic separation of volatile compounds on a carrier gas with high-quality capillary columns	Most widely established analytical method for fatty acids with available automated instruments commercially available.	Cannot analyse involatile compounds. Thus derivitisation of fatty acid methyl esters to analyse triglycerides is required	Seppänen-Laakso *et al*., 2002; Edwards, 2007.

Several methodologies could be used for the quantification of fatty acid composition in oils (Wang *et al*., 2015; Xu *et al*., 2015). The classical methods for fatty acid content quantification are time-consuming, laborious, tedious gravimetric methods which also require a relatively large number of samples, chemical reagents and glassware (Mathias *et al*., 1987; Zhang & Lee, 1997; Xu *et al*., 2015). To quantify individual fatty acids in fish oil samples, high-performance liquid chromatography (HPLC), thin layer chromatography (TLC), and gas chromatography (GC) are frequently used techniques (Zhang & Lee, 1997; Cascant *et al*., 2018; Xu *et al*., 2015). However,

these methods involve substantial sample preparation steps to separate the lipid fraction before analysis (Xu *et al*., 2015). Table 5.1 gives an overview of these methods including their respective principles, advantages, as well as disadvantages.

TLC and HPLC are regularly used in many separations and identification of individual lipids in foods (Cascant *et al*., 2018). TLC is a sequential procedure that is achieved on a stationary phase due to polarity differences of the analytes (Fuchs *et al*., 2011; Cascant *et al*., 2018). HPLC is a separation method that is achieved on a stationary phase under high pressure by elution with different solvents (Fuchs *et al*., 2011). GC has been the method of choice in fatty acid analysis for over 50 years (Lehotay & Hajšlová, 2002; Seppänen-Laakso *et al*., 2002; Edwards, 2007) and has become a widely adopted highly applicable tool in micro-scale analytical work in a number of fatty acid research areas (Seppänen-Laakso *et al*., 2002). Gas chromatography necessitates working at high temperature (Bystrickà & Ďuračkovà, 2016). This may not be appropriate for accurately quantifying thermally labile PUFA (Mehta *et al*., 1998).

NIR spectroscopy has been recognised as a powerful analytical technique for fatty acid determination (McClure, 1994; Velasco *et al*., 2004). Combined with chemometrics, NIR spectroscopy can provide an alternative to the chromatographic techniques that are currently used for fatty acid determination once an appropriate model has been developed (Cascant *et al*., 2013). NIR spectroscopic methods allow for rapid, non-destructive and non-hazardous determination of various constituents in food, agricultural and other products (Osborne & Fearn 1986; Williams & Norris 1987). Quite a few studies on the use of NIR spectroscopy to analyse animal fats and oils were found in literature, but none of them related to the use of NIR spectroscopy to analyse fatty acid composition (Cozzolino *et al*., 2005). Earliest studies on the use of NIR spectroscopy to determine fatty acids in vegetable oils were first reported by Holman and Edmondson in 1956, followed by the analysis of patterns of fatty acids in both fats and oils by Sato *et al*. (1991) and Sato *et al*. (1999). NIR spectroscopy in combination with multivariate techniques provides a powerful tool for monitoring a variety of quality control processes in the food industry and is gaining interest (Cozzolino *et al*., 2005).

Partial least squares (PLS) regression is a projection of the NIR spectral data that uses response information for building models (Garcia-Jares & Medina, 1997; Dahlman, 2012). It is useful in describing the relationship between multiple response variables and predictors (Jun *et al*., 2009; Dahlman, 2012). This results in reduced spectral data without discarding valuable information (Osborne *et al*., 1993). This study aimed at building calibration models using PLS regression to quantitatively compare the predicted values with reference data obtained by GC.

5.2 Material and methods

5.2.1 Samples used for model building

Sardine samples (n = 64) used in this study were caught in the west coast of South Africa during June and July 2017 from commercial purse seiners. After fishing, the sardine samples were covered with blue polyethylene bags in boxes and immediately frozen on board. After unloading, fish samples were transported to the Department of Agriculture, Forestry and Fisheries (DAFF), Cape Town, South Africa. SA sardine samples were transported to the laboratories of the Department of Food Science and Technology (DFST), Cape Peninsula University of Technology (CPUT) for subsequent sample preparation. Upon arrival at DFST laboratories, the ungutted sardine samples were individually packed in vacuum bags and stored in a freezer between -17 and -20 °C until subjected to NIR spectral acquisition and fatty acid analysis.

5.2.2 Sample preparation

Samples were thawed overnight in a refrigerator at 4°C before fatty acid analysis. Fish samples were gutted and homogenised using a FOSS (Hillerød, Denmark) Knifetec 1095^{TM} DK-3400 sample mill at 20 000 rpm per 10 s.

5.2.3 Fatty acid measurement

The Bligh and Dyer (1959) method was used for determining fatty acids composition. Fat was extracted from 1g of a homogenised sample using chloroform/methanol/water mixture. Fat extracts were converted into fatty acid methyl esters (FAME) using hydrochloric acid and then analysed by gas chromatography (Agilent Technologies 7890A GC system, Wilmington, USA). A fused capillary column HP88 (100m x 0.25 mm x 0.25 µm) was used, and the temperature program was as follows: increased from 100°C to 240°C at 3°C/min held at 100°C for 4 min. The carrier gas was nitrogen at ten psig, and detection was performed with a flame ionisation detector at 325°C. A programmed temperature vaporiser injector was used in the split mode (50:1) and was heated from 45°C to 250°C at 15°C/min. Peaks corresponding to fatty acid methyl esters (FAME) were identified by comparing their retention times with those of standard (SIGMA-ALDRICH Inc. Darmstadt, Germany) mixtures. Peak areas were automatically integrated. The content of each fatty acid was expressed as percentage weight of total fatty acids.

5.2.4 NIR spectral acquisition

The NIR reflectance spectra measurements of SA sardine samples (n=64) were acquired over the wavelength from 908 to 1680 nm using a hand-held Viavi's MicroNIR 1700 (Viavi Solutions Inc., Milpitas, CA, USA) spectrophotometer. The external white reference used was a diffuse reflectance standard. The samples (n=64) were individually placed in a custom-made hollowed-out Teflon (PFTE) disk (Maizey's (Pty) Ltd, Kuilsriver, South Africa) that completely covered the contactable collar of the MicroNIR spectrophotometer upon scanning. Each scan was the average of 64 individual scans (scanning parameter). MicroNIR software (Viavi Solutions Inc., Milpitas, CA, USA) was used to perform the spectroscopic measurements. All samples were measured in duplicate, and the Teflon disc was cleaned between consecutive samples. All spectral data were saved in Microsoft Excel format for further analysis in The Unscrambler software (Camo ASA, Oslo, Norway) version 10.4.

5.3 Results and discussion

The fatty acid composition, expressed as the percent saturated fatty acids (%SFA), mono-unsaturated fatty acids (%MUFA), polyunsaturated fatty acids (%PUFA), as well as the total fatty acids (%Total FA) of the SA sardine samples is shown in Table 5.2. These results were obtained from homogenised sardine samples (n = 64) using a standard GC method. All the fatty acid constituents varied widely, with the total FA composition varying the least as can be seen in Table 5.2. As a visual display of the range and distribution of the respective fatty acid compositions, histograms are showed (Figs. 5.1 a to d).

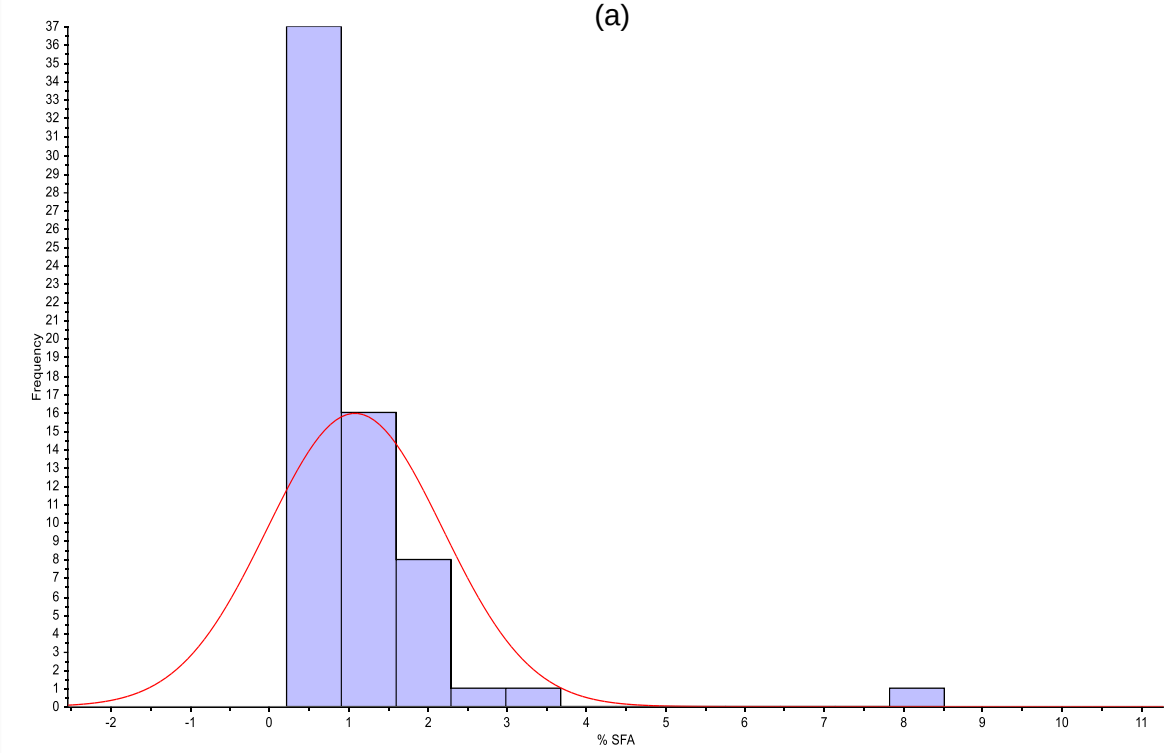
Table 5.2: Statistical overview of the fatty acid analysis of sardine samples (all units in %)

	Mean	**Minimum**	**Maximum**	**SD**
%SFA	1.08	0.22	8.52	1.10
%MUFA	2.44	0.06	9.59	2.95
%PUFA	0.96	0.12	6.73	1.06
%Total FA	4.48	0.65	10.88	2.73

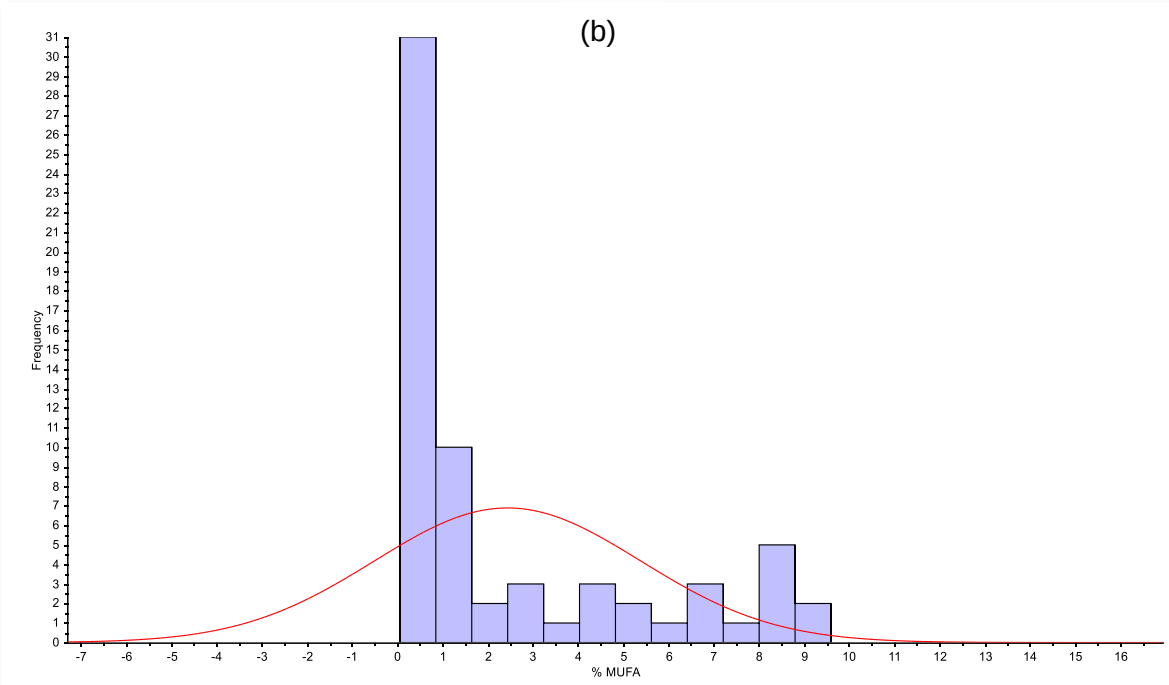
SFA: saturated fatty acid; MUFA: mono-unsaturated fatty acid; PUFA: polyunsaturated fatty acid; TFA: total fatty acids.

SD: standard deviation

(a)



(b)



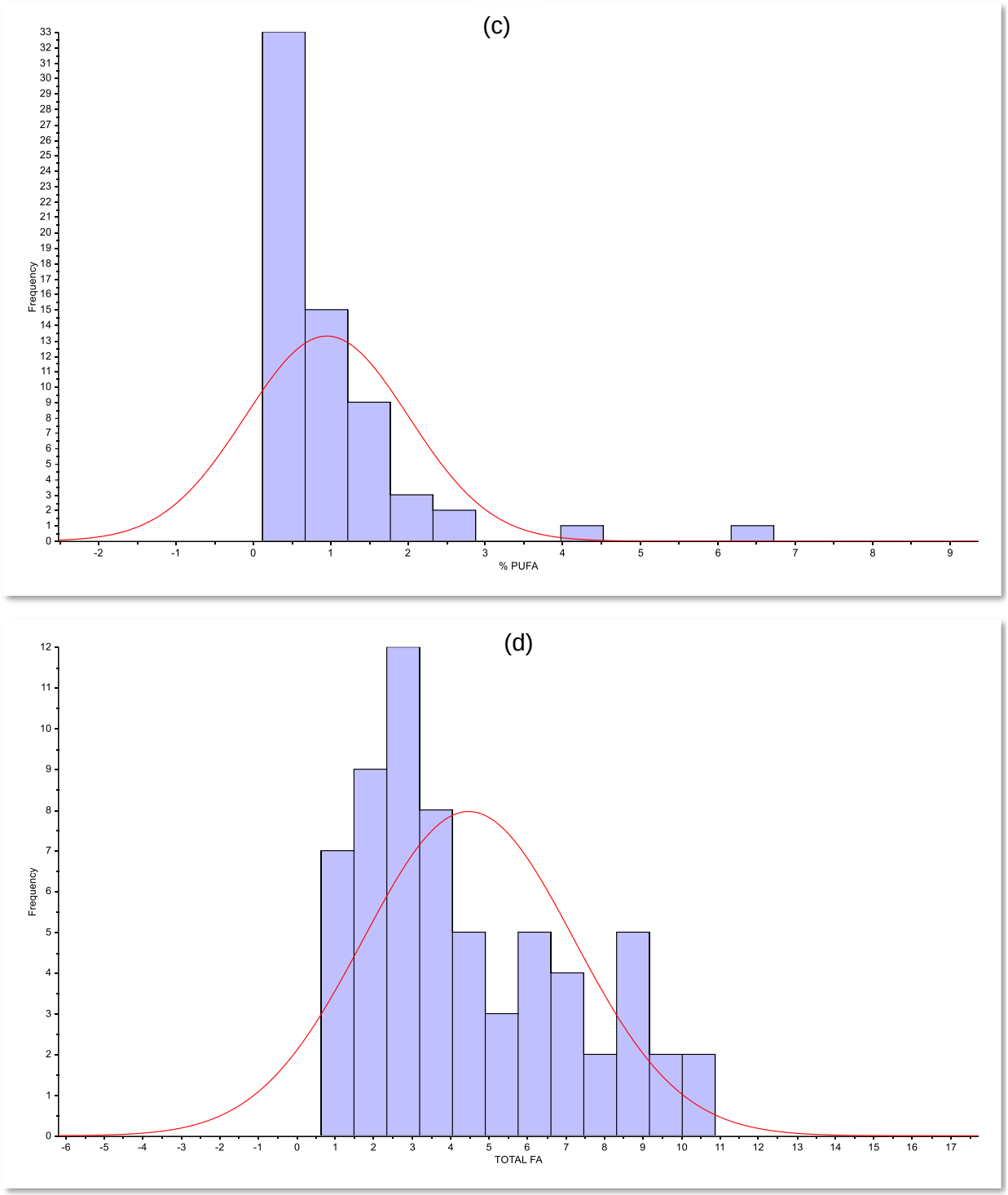
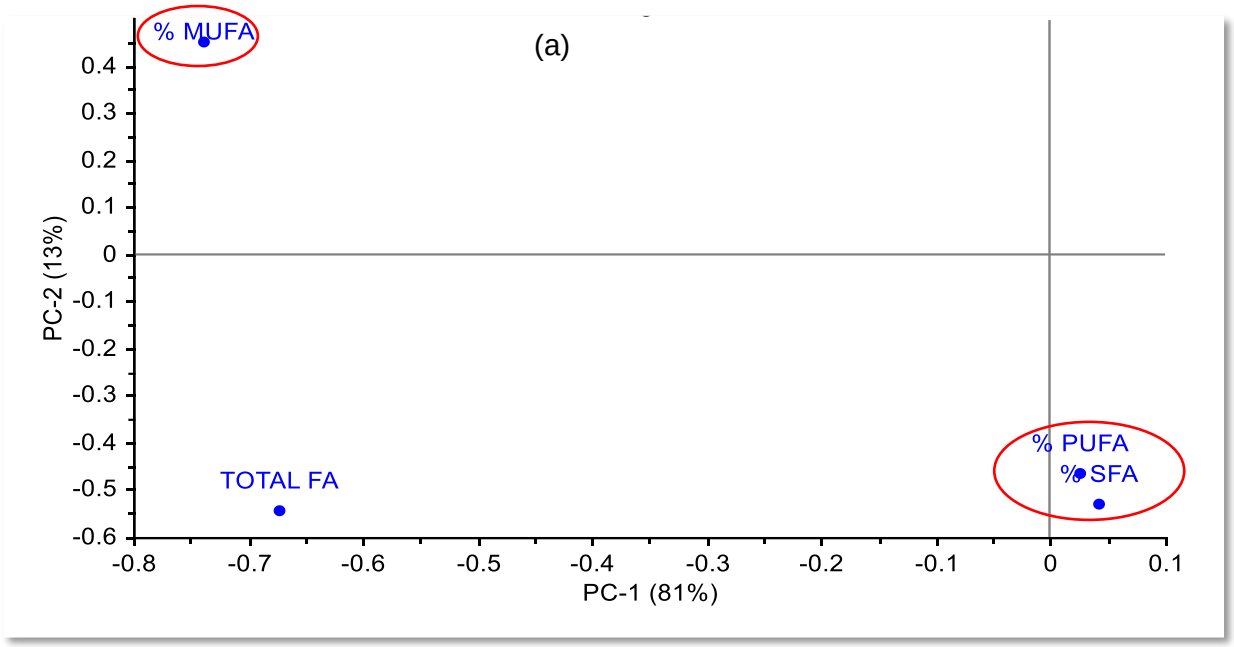


Figure 5.1: Histograms indicating the distribution (or range) of the respective concentrations of the fatty acids (a) %SFA, (b) %MUFA, (c) %PUFA and (d) %Total FA.

Using the NIR spectral data, principal component analysis (PCA) was computed with the four fatty acid constituents showed on the loadings plot Fig. 5.2 a. As would be expected, the MUFA were found in the quadrant opposite that of the PUFA and the SFA (Quadrant I and III). The division or separation was due to the variance found in the second principal component (PC 2). A closer look at the loadings concerning relevant wavelengths, it was seen that the loading line plot of PC 1 (Fig. 5.2 b) indicated towards the wavelength range around 1037 nm that was associated with oil (Osborne *et al*., 1993). The apparent difference in chemical structure between mono- and polyunsaturated fatty acids was confirmed.



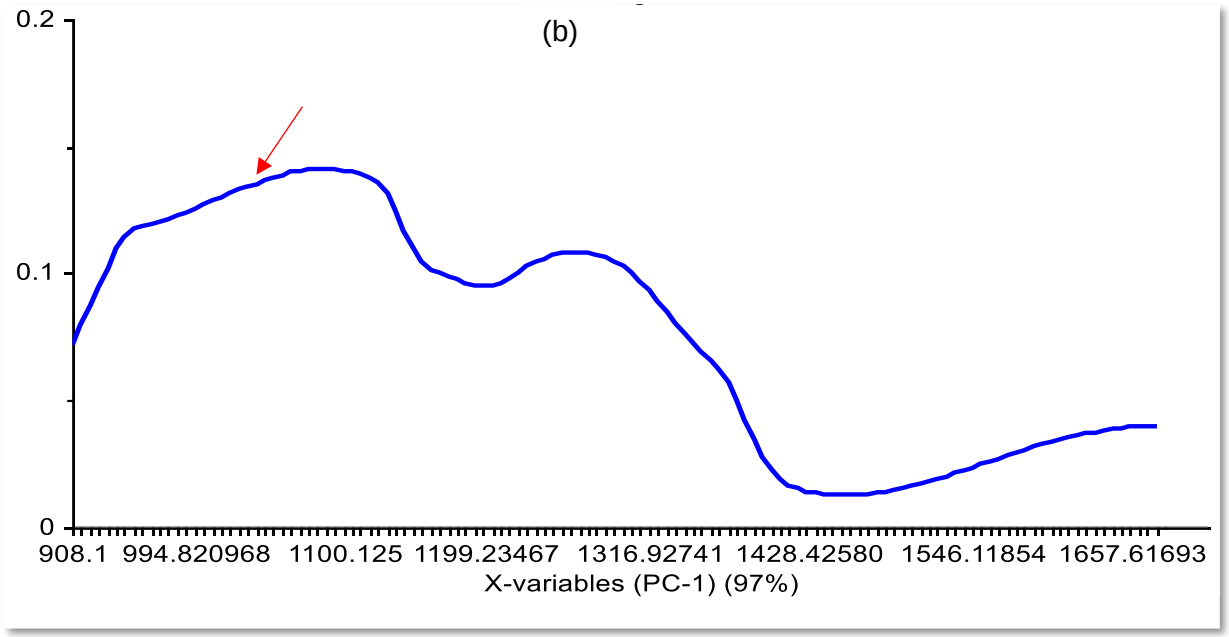


Figure 5.2: (a) A PCA loading plot showing the differences in chemical composition between the different fatty acids, and (b) the loading line plot indicating that the main variance is associated with oil (1037 nm).

Additionally, while examining the PCA model, the Influence plot (Fig. 5.3) indicated that three outliers (samples that should be excluded from the model) were in fact present. Therefore, samples 36, 108 and 118 were deleted from the sample set. The remaining samples (n = 61) were used for the construction of the PLS regression model.

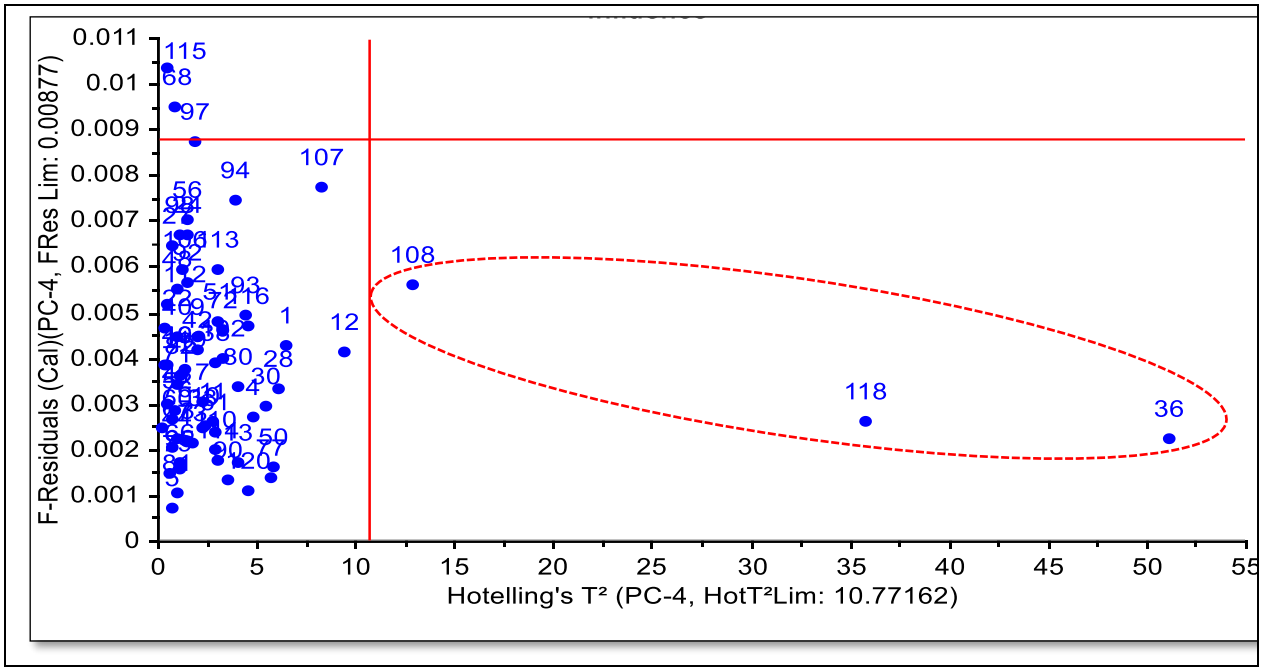


Figure 5.3: A PCA influence plots for the fatty acid data.

Table 5.3: Descriptive statistics of the calibration (n = 45), as well as validation (n = 16) data sets of the SFA, MUFA, PUFA and Total FA % of the SA sardine samples

Samples	Calibration set				Validation set			
	Mean (%)	Min (%)	Max (%)	SD (%)	Mean (%)	Min (%)	Max (%)	SD (%)
% SFA	0.97	0.22	2.99	0.55	1.03	0.22	2.5	0.65
% MUFA	2.32	0.05	9.21	2.99	2.86	0.13	9.60	3.09
% PUFA	0.82	0.13	2.46	0.63	0.79	0.12	2.16	0.65
% Total FA	4.12	0.65	10.34	2.58	4.69	1.14	10.89	2.79

n: number of samples; min: minimum; max: maximum; SD: standard deviation; SFA: saturated fatty acid; MUFA: mono-unsaturated fatty acid; PUFA: polyunsaturated fatty acid; Total FA: total fatty acids.

Calibration equations were calculated on transformed spectra. For the PLS regression calibrations predicting %SFA, %MUFA, %PUFA and the %Total FA, mean-centered, MSC along with S-G second derivative smoothing was applied to the spectra. Visually presented, the spectra were transformed from raw spectra in (Fig. 5.4 a) to the pre-treated spectra as depicted in Fig. 5.4 b.

Calibration and validation results for the prediction of the different fatty acids constituents of the homogenised SA sardine samples are reported in Table 5.4. A reasonable correlation and

relatively lowest prediction error were obtained for the %SFA calibration. The Explained variance plot (Fig. 5.5 a) indicated the use of only four factors and not morel. This resulted from doing a full-cross validation step. In Fig. 5.5 a, the blue line represents the calibration samples and the red line the full-cross validation samples. Using five factors could also have been considered, but due to the fact that the calibration model only consisted of forty-five samples, the choice of using only four factors was made. When considering the Predicted vs. Reference plot (Fig. 5.5 b), a reasonable coefficient of determination (or R^2_c) of 0.64 could be seen. The error associated with the prediction using the calibration set was 0.33% for the SFA. The prediction results of the validation step, using the independent sample set (validation set), are always worse than that of the calibration set. None the less, these results should be presented, because they are probably more realistic results than that of the calibration statistics. In this case, the correlation coefficient for the validation set was $R^2_v = 0.48$ and the error of prediction (SEP) was 0.45%. Using this SEP result, the RPD value was calculated in order to put the prediction into perspective with regards to the conventional analytical method. For this calculation, the SD associated with the analytical method, in this case the GC method to determine the %SFA for the validation set was 0.65% (as can be seen in Table 5.3). Therefore, the RPD = 0.65% / 0.45% = 1.44 (Table 5.4). The low RPD value indicated that the calibration was not effective, as a value above 5 indicates towards a successful calibration. When comparing the prediction results of SA sardine fat to the results of Cascant *et al*. (2018) for salmon (*Salmo salar*) with an RPD of 1.39 obtained for (MUFA), 2.41 PUFA and 1.62 for SFA, NIR spectroscopy can be seen as a method with low predictive ability for fatty acids.

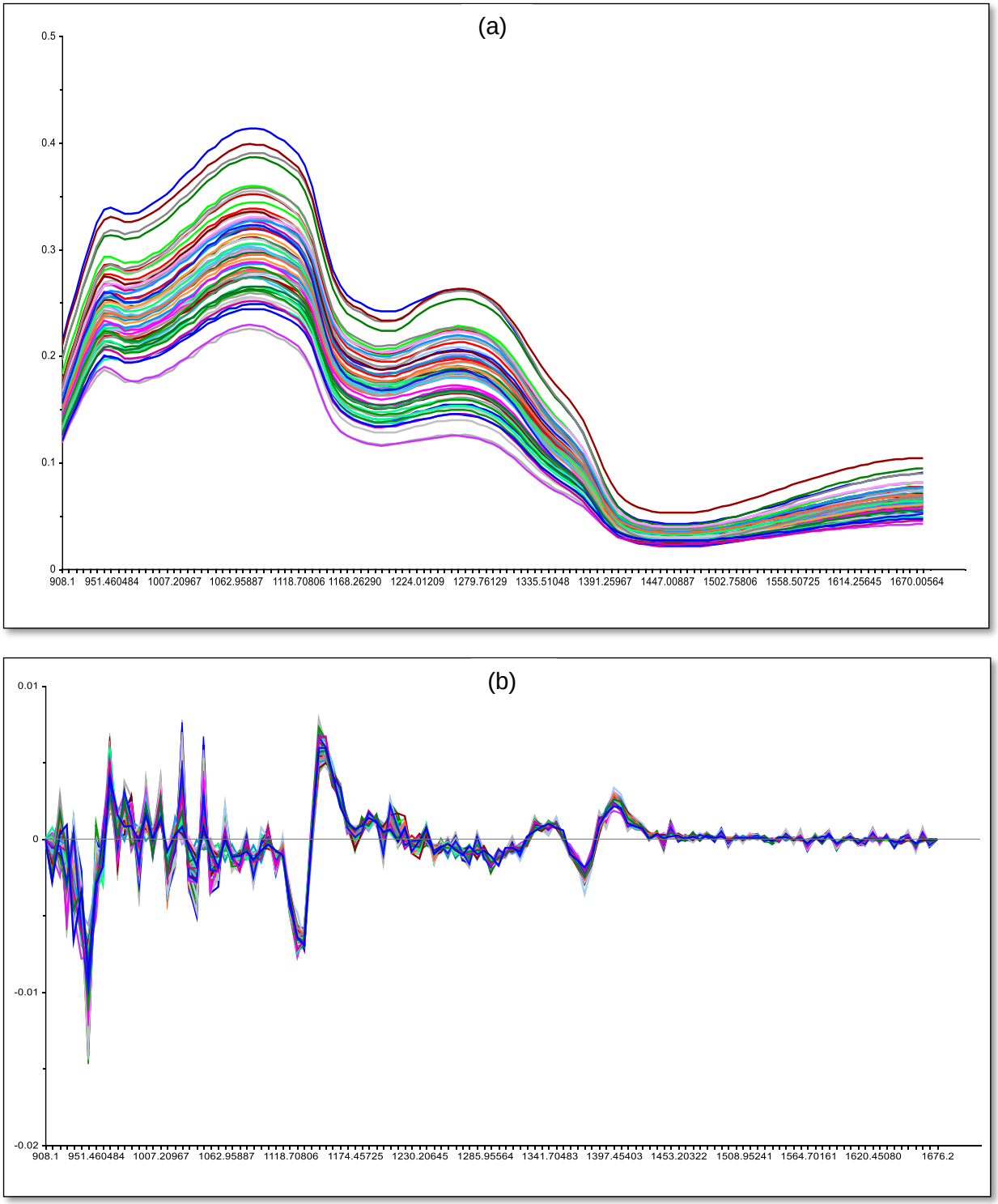


Figure 5. 4: (a) Original NIR spectra and (b) transformed spectra, using a combination of pre-processing options, i.e. MSC and S-G second derivative smoothing.

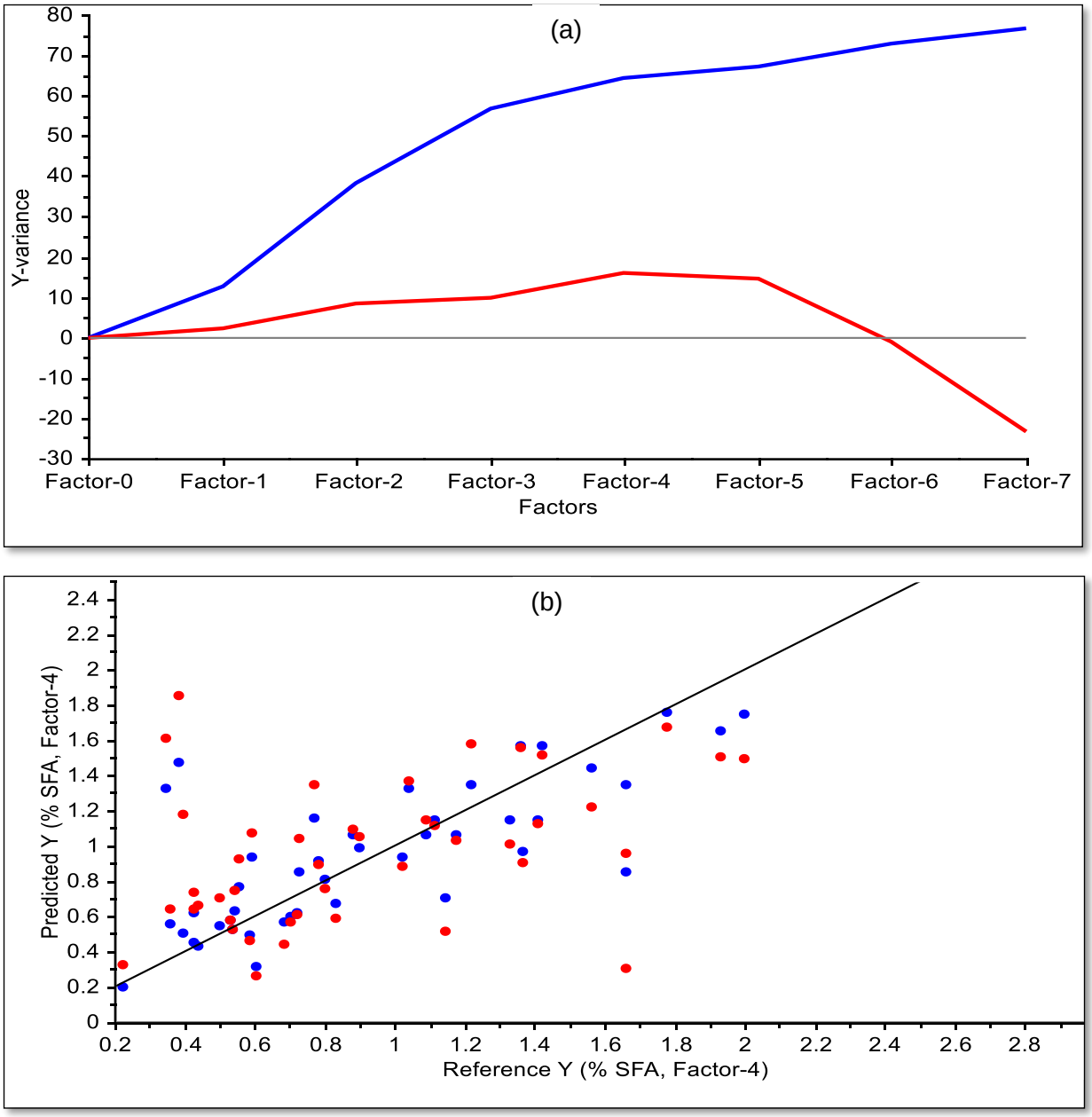


Figure 5.5: NIR spectroscopy calibration plots for %SFA: (a) an Explained variance plot, and (b) a Predicted vs. Reference plot.

Even when applying the extensive pre-processing as in the previous calibration, the prediction results of the %MUFA were very poor. Very little correlation was found between the predicted and

reference values (R^2_c = 0.38) (Fig. 5.6), and the SEC was 2.33% when using four factors (Table 5.4). There was no need to do a further validation step.

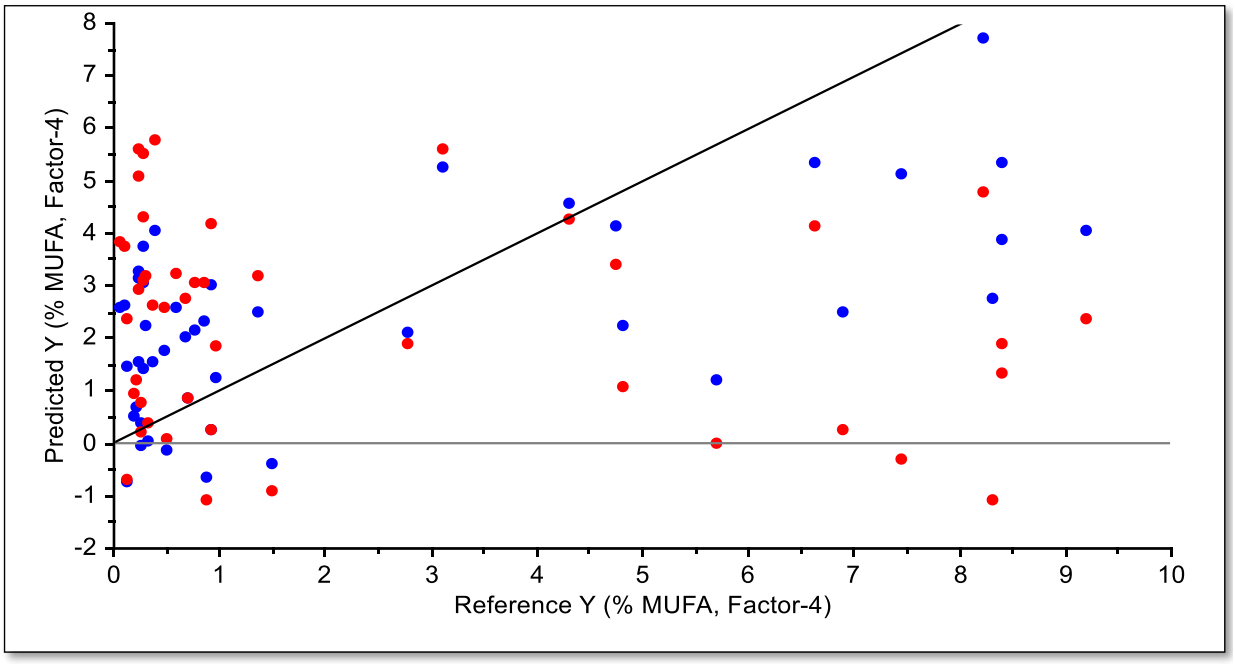


Figure 5. 6: The NIR spectroscopy Predicted vs Reference plot for %MUFA.

The prediction results for %PUFA indicated towards a better calibration than that of %MUFA. Again using four factors and the same pre-processing as previously described for the two calibrations, the standard error of calibration was 0.39% with a regression coefficient of 0.60 (Figure 5.7; Table 5.4). For the validation set the SEP was 0.44% and the R^2_v = 0.48 (Table 5.4). The SD of the validation set divided by the SEP resulted in an RPD value of 1.47 (Table 5.4). Again, the calibration was not effective. A larger sample set with calibration samples being in the vicinity of 100 samples can result in a better calibration due to the data set containing greater chemical and spectral variation that must be presented in a robust calibration model (Davies & Grant, 1987; Au *et al*., 2020).

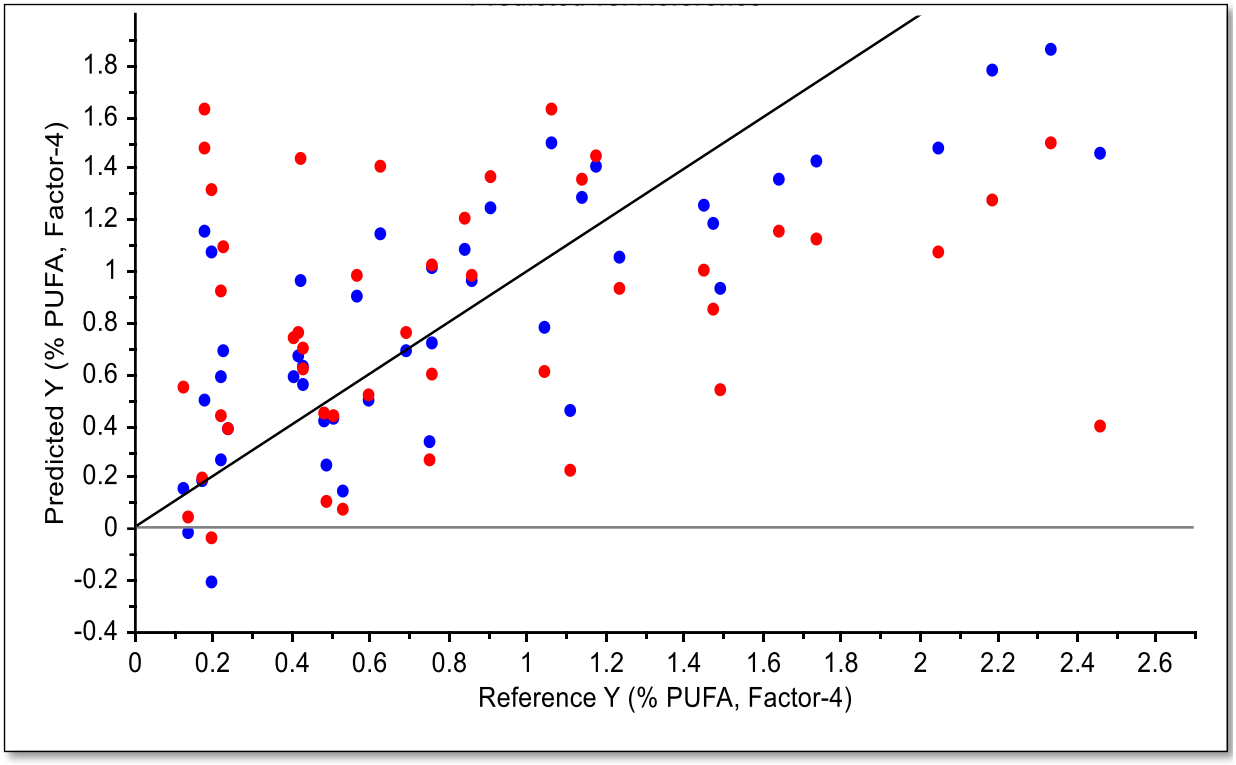


Figure 5. 7: The NIR spectroscopy Predicted vs. Reference plot for %PUFA.

Lastly, the PLS regression calibration for the prediction of the percentage total fatty acids (TFA) did not prove to be effective at all. Already from the Predicted vs. Reference plot of the calibration set, the very poor determination coefficient results of 0.37 showed no real predictive ability (Fig. 5.8; Table 5.4). The error associated with this prediction was 2.03% (Table 5.4). The usual validation step was again not followed, as it was evident that NIR did not seem to be able to predict well between the SA sardine samples based on %TFA.

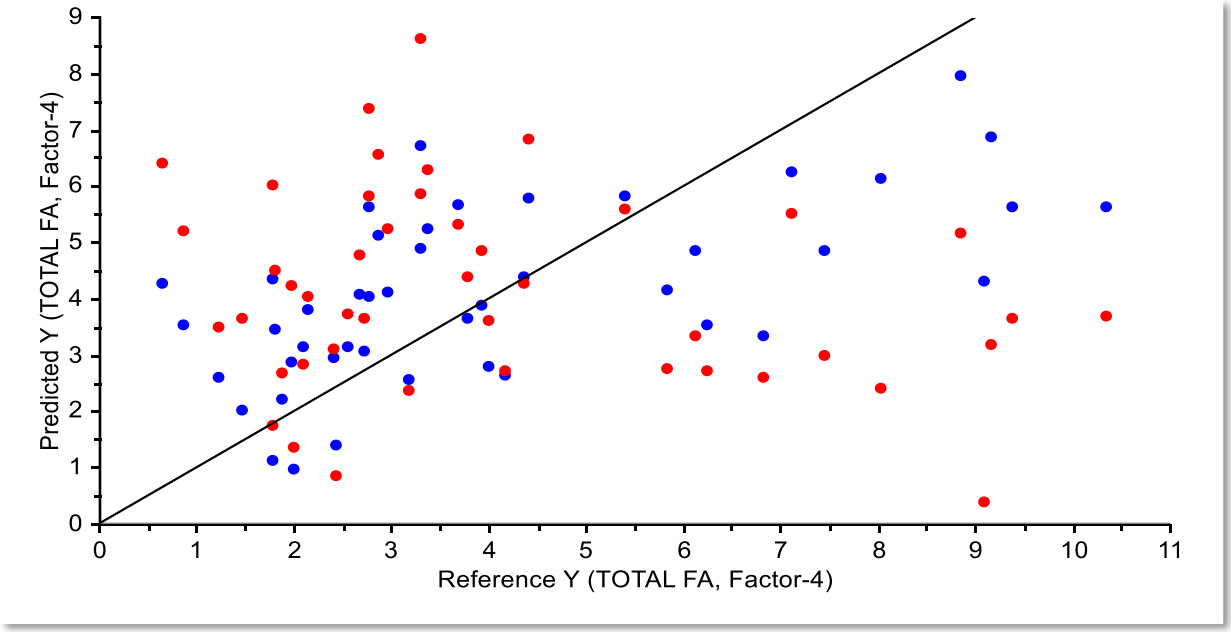


Figure 5. 8: The NIR spectroscopy Predicted vs. Reference plot for %TFA.

Table 6: Prediction of fatty acid composition corresponding to homogenised SA sardine samples after applying MSC and second derivative S-G pre-processing to the spectra

	Calibration			Validation		
	Number of factors	**RMSECV** (%)	**R^2_c**	**RMSEP** (%)	**R^2_v**	**RPD**
%SFA	4	0.33	0.64	0.45	0.48	1.44
%MUFA	4	2.33	0.38	Not relevant	-	-
%PUFA	4	0.39	0.60	0.44	0.48	1.47
%Total FA	4	2.03	0.37	Not relevant	-	-

RMSEC: Root mean square error of calibration; R^2_c: coefficient of determination of the calibration; RMSEP: root mean square error of the prediction; R^2_v: coefficient of determination of the validation; RPD: Ratio Performance Deviation; SFA: saturated fatty acid; MUFA: mono-unsaturated fatty acid; PUFA: polyunsaturated fatty acid; TFA: total fatty acids.

5.4 Conclusions

Knowledge of the quantities of fatty acids in SA sardines is important for the development of sardine products and to assess the nutritional value thereof. Conventionally, fatty acids are determined using methods such as HPLC, TLC and GC. In this study, using GC, the %SFA, %MUFA, %PUFA, as well as the %TFA of South African sardines were determined. In addition, acquired NIR spectra following PLS regression model building was applied on the same sardines. The predictability of the fatty acid content of sardines on the basis of %SFA and %PUFA, as obtained from GC analysis was unsatisfactory. However, %MUFA and %TFA had very little correlation between predicted and reference values, and it was evident that NIR spectroscopy could not predict these constituents to a satisfactory level. These respective calibration results suggested that fatty acids could be not be predicted using the fast and low cost NIR spectroscopy technique. When considering the existing usefulness of NIR spectroscopy in the food industry, further research into this specific application is therefore warranted in the fish industry.

References

Au, J., Youngentob, K.N., Foley, W.J., Moore, B.D. & Fearn, T. (2020). Sample selection, calibration and validation models developed from a alrge data set of near infrared spectra of tree leaves. *Journal of Near Infrared Spectroscopy*, **28**(4), 186-203.

Bagthasingh, C., Aran, S.S., Vetri, V. & Innocen, A. (2016). Seasonal variation in the proximate composition of sardine (*Sardinella gibbosa*) from Thoothukudi coast*. Indian Journal of Geo-Marine Science*, **45**, 800–806.

Bandarra, M.N., Batiota, I., Nunes, M.L., Empis, J.M. & Christie, W.W. (2006). Seasonal changes in lipid composition of sardine (*Sardina pilchardus*). *Journal of Food Science*. **62**(1), 40-42

Bligh E.G. & Dyer W.J. A rapid method of total lipid extraction and purification**.** *Canadian Journal of Biochemistry and Physiology 1959.* **37**(8), 911-917

Bulla, M.K., Simionato, J.I., Matsushita, M., Garcia Coro, F.A., Shimokomaki, M., Visentainer, J.V. & de Souza, N.E. (2011). Proximate composition and fatty acid profile of raw and roasted salt-dried sardines (*Sardinella brasiliensis*). *Food and Nutrition Sciences*, **2**, 440-443.

Bystrickà, Z. & Ďuračkovà, Z. (2016). Gas chromatography determination of fatty acids in the human erythrocyte membranes – A review. *Prostaglandins, Leukotrienes and Essential Fatty Acids,***115**, 35-40.

Capanio, F., Lestingi, A., Summo, C., Bilancia, M.T. & Laudadio, V. (2004). Chemical characteristics and lipid fraction quality of sardines (*Sardina pilchardus W*.): influence of sex and length. *Journal of Applied Ichthyology*, **20**, 530-535.

Cascant, M.M., Breil, C., Fabiano-Tixier, A.S., Chemat, F., Garrigues, S. & de la Guardia, M. (2018). Determination of fatty acids and lipid classes in salmon oil by near-infrared spectroscopy. *Food Chemistry*, **239**, 865-871.

Çelik, M., Diler, A. & Küçükgülmez, A. (2005). A comparison of the proximate compositions and fatty acid profiles of zander (*Sander lucioperca*) from two different regions and climatic conditions. *Food Chemistry*, **92**, 637–641.

Chanmugam, P., Boudreau, M. & Hwanm, D.H. (1986). Differences in the n-3 fatty acid content in pond-reared and wild fish and shellfish. *Journal of Food Science*, **51**(6), 1556-1557.

Coetzee, J.C., Van der Lingen, C.D., Hutchings, L. & Fairweather, T.P. (2008). Has the fishery contributed to a major shift in the distribution of South African sardine? *ICES Journal of Marine Science*, **65**, 1676-1688.

Cozzolino, D., Murrya I., Chree, A., Scaife, J.R. (2005). Multivariate determination of free fatty acids and moisture in fish oils by partial least squares regression and near-infrared spectroscopy. *LWT*, **38**, 821-828.

Davies A.M.C., Grant. A. (1987). Review: Near infrared analysis of food. *International Journal of Food Science & Technology*, **22**, 191–207.

Eder, K. (1995). Gas chromatographic analysis of fatty acid methyl esters. *Journal of Chromatography B: Biomedical Applications*, **671**, 113-131.

Edwards, W.P. (2007). Analytical chemistry. In: The Science of Bakery Products (edited by W.P Edwards). p.138. United Kingdom: Royal Society Chemistry Publishers.

Fuchs, B., Süß, R., Teuber, K., Eibisch, M. & Schiller, J. (2011). Lipid analysis by thin layer chromatography- A review of the current state. *Journal of Chromatography A*, **1218**, 2754-2774.

Gámez-Meza, N., Higuera-Ciapara, I., Calderon de la Barca, A.M., Vázquez Moreno, L., Noriega-Rodrígueza, J. & Angulo-Guerrero, O. (1999). Seasonal variation in the fatty acid composition and quality of sardine from Sardinops sagax caeruleus of the Gulf of California. *Lipids*, **34**(6), 639-642.

Gockse, M.A., Tasbozan, O., Celik, M. & Tabakoglu, S.S. (2004). Seasonal variation in proximate and fatty acid compositions of common female sole (*Solea solea*). *Food Chemistry*, **88**, 419-423.

Hardy, R. & Keay, J.N. (1972). Seasonal variation in the chemical composition of Cornish mackerel*, Scomber scombrus* (L.) with detailed reference to the lipids. *Journal of Food Technology*, **7**, 125-137.

Holman, R.T. & Edmondson, P.R. (1956). Near-infrared spectra of fatty acids and some related substances. *Analytical Chemistry*, **28**(10), 1533-1537.

Hussein, J.S. (2013). Cell membrane fatty acids and health. *International Journal of Pharmaceutical Sciences*, **5**, 38-46.

Jabeen, F. & Chaudhry, A.S. (2011). Chemical compositions and fatty acid profiles of three freshwater fish species. *Food Chemistry*, **125**, 991–996.

Jun, C, Lee, S., Park, H. & Lee, J. (2009). Use of partial least squares regression for variable selection and quality prediction. *International Conference on Computers & Industrial Engineering*, 1302-1307.

Khitouni, I.K., Mihoubi, N.B., Bouain, A. & Rebah, F.B. (2014). Seasonal variation of the chemical composition, fatty acid profiles and mineral elements of *Diplodus Annularis* (Linnaeus, 1758) caught in the Tunisian coastal water. *Journal of Nutrition Research*, **2**(6), 306-311.

Lehotay, S.J. & Hajšlová, J. (2002). Application of gas chromatography in food analysis. *Trends in Analytical Chemistry*, **21**(9&10), 686-697.

Love, R.M. (1980). The chemical biology of fishes. p. 387. London: Academic Press.

Love, R. M., (1988). The Food Fishes. Their intrinsic variation and practical implications. Pp. 25-86. London: Farrand Press.

Luzia, L.A., Sampaio, G.R., castellucci, C.M.N. & Torres, A.F.S. (2003). The influence of season on the lipid profiles of five commercially important species of Brazilian fish. *Food Chemistry*, **83**, 93-97.

Mathias, J.A., Williams, P.C. & Sobering, D.C. (1987). The determination of lipid and protein in freshwater fish using near infrared reflectance spectroscopy. *Aquaculture*, **61**, 303-311.

Mehta, A., Oeser, A.M; Carlson, M.G. (1998). Rapid quantification of free fatty acids in human plasma by high-performance liquid chromatography. *Journal of Chromatography* B, **719**, 9-23.

McClure, M.F. (1994). The giant is running strong. *Analytical Chemistry*, **66**, 43-53.

Mraz, J. & Pickova, J. (2011). Factors influencing fatty acid composition of common carp (*Cyprinus carpio*) muscle. *Neuroendocrinology Letters*, **32**(2), 3-8.

Nunes, M.L.; Cardinali, M.; Mendes, R.; Campos, R.M.; Bandarra, N.M.; Lourenco, H.M.; Jerome, M. (1991). Effect of season and ice storage on proteins and lipids of sardine (*Sardina*

pilchardus) minces and surimi. In: Quality assurance in the fish industry (edited by H.Huss; M. Jakobsen & J. Liston). Pp. 63-72. Amsterdam: Elsevier.

Osborne, B.G. & Fearn, T. (1986). *Near-Infrared Spectroscopy in Food Analysis*. Longman Scientific & Technical: Longman House, Essex.

Osborne, B.G., Fearn, T. & Hindle, P.H. (1993). Practical NIR Spectroscopy with applications in food and beverage analysis, 2nd edition, Pp. 1-220. England: Longman Group UK Limited.

Rahman, S.A., Huah, T.S., Hassan, O. & Daud, N.M. (1995). Fatty acid composition of some Malaysian freshwater fish. *Food Chemistry*, **54**, 45-49.

Rodrigues, B.L, Canto A.C.V.C.S., Costa, M.P., Silva F.A., Ma´rsico E.T., Conte-Junior C.A (2017) Fatty acid profiles of five farmed Brazilian freshwater fish species from different families. *PLoS ONE*, **12**(6), 1-15.

Sato, T., Kawano, S. & Iwamoto, M. (1990). Fatty acid composition in oil and NIR spectral pattern. In: *Third International Conference on Near Infrared Spectroscopy*, Pp. 242-248. Belgium: Gemblaux.

Sato, T., Kawano, S. & Iwamoto, M. (1991). Near-infrared spectral patterns of fatty acid analysis from fats and oils. *Journal of the American Oil Chemists Society*, **68**, 827-833.

Schmitt-Rozieres, M., Deyris, V., Comeau, L. (2000). Enrichment of polyunsaturated fatty acids from sardine cannery effluents by enzymatic selective esterification. *Journal of the American Oil Chemists Society*, **72**(3), 329-332.

Seppänen-Laakso, T., Laakso, I. & Hiltunen, R. (2002). Analysis of fatty acids by gas chromatography and its relevance to research on health and nutrition. *Analytica Chimica Acta*, **465**, 39-62.

Shirai, N., Terayam, M. & Tekada, H. (2002). Effect of season on the fatty acid composition and free amino acid of the sardine *Sardinops melanostictus*. *Comparative Biochemistry and Physiology Part B*, **131**, 387-397.

Syme, D. A, &R.E Shadwick, R.E.(2011) Red muscle function in stiff-bodied swimmers: there and almost back again. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **366**(157), 1507-1515.Tarley, C.R.T., Visentainer, J.V., Matsushita, M. & de Souza, N.E. (2004). Proximate composition, cholesterol and fatty acid profile of canned sardines (*Sardinella brasiliensis*) in soybean oil and tomato sauce. *Food Chemistry,* **88**, 1-6.

Ural, M.S., Çalta, M. & Parklak, A.E. (2017). The comparison of fatty acids, fat-soluble vitamins and cholesterol in the muscle of wild caught, cage and pond reared rainbow trout (*Oncorhynchus mykiss* W., 1792). *Iranian Journal of Fisheries Sciences*, **16**(1), 431-440.

Van der Lingen, C.D. (2002). Diet of sardine *Sardinops sagax* in the southern Benguela upwelling ecosystem. *South African Journal of Marine Science*, **24**(1), 301-316.

Vasconcellos, M. (2003). Analysis of harvest strategies and inforrmation needs in the purse seine fishery for the Brazilian sardine. *Fisheries Research*, **59**(3), 363-378.

Velasco L., Perez-Vich B., Fernandez-Martinez J.M. (2004). Use of near-infrared reflectance spectroscopy for selecting for high stearic acid concentration in single husked achenes of sunflower. *Crop Science*, **44**,93-97

Wang, J., Wu, W., Wanga, X. & Wu, F. (2015). An effective GC method for the determination of the fatty acid composition in silkworm pupae oil using a two-step methylation process. *Journal of the Serbian Chemical Society*, **80**(1), 9-20.

Wang, Y.L., Miller, L.A., Perren, M. & Addis, P.B. (1990). ω−3 fatty acids in Lake Superior fish. *Journal of Food Science*, **55**(1), 71-73.

Wiiliams, P.C. & Norris, K. (1987). *Near Infrared Technology in the Agricultural and Food Industries*, 2nd Edition. American Association of Cereal Chemist: St Paul, MN.

Xu, J., Riccioli, C. & Sun, D. (2015). An overview of nondestructive spectroscopic techniquesfor lipid and lipid oxidation analysis in fish products. *Comprehensive Reviews in Food Science and Food Safety*, **14**, 466-477.

Yeşilayer, N. & GenÇ, N. (2013). Comparison of proximate and fatty acid compositions of wild brown trout and farmed rainbow trout. South African *Journal of Animal Science*, **43**(1), 89-97.

Zamaria, N. (2004). Alteration of polyunsaturated fatty acid status and metabolism in health and disease. *Reproduction Nutrition Development Journals*, **44**, 273-282.

Zhang, H. & Lee, T. (1997). Rapid near-infrared spectroscopic method for the determination of free fatty acid in fish and its application in fish quality. *Journal of Agricultural and Food Science*. **45**, 3515-3521.

Zlatanos, S. & Lastaridis, K. (2007). Seasonal variation in the fatty acid composition of three Mediterranean fish – sardine *(Sardina pilchardus*), anchovy (*Engraulis encrasicholus*) and picarel (*Spicara smaris*). *Food Chemistry*, **103**, 725-728.

6 CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSIONS

Consumer trends towards health and nutrition have led to an enormous increase in the consumption of fish and fishery products over the years (Huang *et al*., 2014). Concurrently, the demand for fish has led to consumers being more concerned about obtaining accurate information about the food they eat, primarily relating to the correspondence of the food contents with what is declared on the label. Therefore, there is a need for comprehensive nutritional information for a large variety of fish and fishery products, both for nutritional labelling and for incorporation into food composition tables for nutritional purposes.

The SA sardines (*Sardinops sagax ocellatus)* commonly known as pilchards, are commercially caught by South Africa's purse-seine fishery along the entire South African coastline from the Orange River on the west coast to KwaZulu Natal (~27°S) on the east coast (Coetzee *et al*., 2008). Sardines are one of the most ecological and economic essential fish species in the Benguela ecosystem and are consumed both locally and internationally.

The assessment of fish condition in SA sardine population can be used as a non-invasive method to discriminate fish according to different fat stages. Morphophysiological indicators have been described and have been used in the assessment of SA sardine condition (De Goede, 2004). Lipid content in fish is known to be a good indicator of the condition of a population (Patterson, 1992). Lipid analysis of *Sardinops sagax* by visual assessment of mesenteric fat has been successfully applied in a study by Van der Lingen and Hutchings (2005). For sustainable management of SA sardine stocks, it is important for the fish canning industry to have data on the sardine lipid content. However, it should be noted that the analytical methods used to determine the proximate composition as well as fatty acids of the sardine cannot be used in a fast-paced, processing environment due to their time consuming, expensive and destructive characteristics (Cozzolino *et al*., 2005). For this purpose, previous studies (Saeed *et al*., 2012) have shown near infrared (NIR) spectroscopy to be a reliable method for predicting fish chemical composition. Grouping of SA sardine samples according to different fat and gonad stages was observed in whole fish samples as opposed to the homogenised sample set using NIR spectroscopy with chemometrics in the form of PCA. The results showed that NIR spectroscopy combined with morphophysiological characteristics could be used as a technique to group whole sardines according to the different fat and gonad stages. For the homogenised sample set, NIR spectroscopy could be used to group samples according to the respective fat stages.

The evaluation of proximate composition in fish is the most important aspect in fish nutrition. The proximate composition of SA sardine was determined using conventional methods such as oven drying for moisture, incineration for ash, combustion for protein and solvent extraction for fat analysis. The proximate composition results of SA sardines were found to be similar to that of other sardine species (Hale, 1984; Feng *et al*., 2012; Suseno *et al*., 2014) but were found to contain higher protein content with little fat content. The deviation in protein and fat contents could be due to many reasons, the main one being sample preparation. The heterogeneity of a sample can affect the proximate results of fish. Collection and preparation of samples must, therefore, be carefully considered to ensure analysis of a homogeneous and representative sample and to obtain accurate results.

The feasibility of near-infrared spectroscopy (NIR) in conjunction with partial least square (PLS) regression to quantitatively predict the proximate composition of SA sardine was evaluated. The obtained proximate results were correlated with the fast, simple and non-destructive NIR spectra acquired using a miniature hand-held microNIR device; this was achieved by building a (PLS) model. PLS models calculated for moisture, ash, fat and protein demonstrated that predictions could not be made with the fast NIR scan. Additionally, the fatty acid composition results of sardines were correlated with the non-destructive NIR spectroscopy. Similarly, the results were used to build PLS models, resulting in fair predictions for saturated fatty acids and polyunsaturated fatty acids (PUFA). Monounsaturated fatty acids (MUFA) and total fatty acids (TFA) had very poor prediction results with very little correlation found between the predicted and reference values.

The predictive quality of these models was very poor, not even good enough to be used as a rough screening method by the pelagic fishery. Different factors might have influenced the predictive quality of the created PLS models. Agricultural products such as fish are are very complex materials, comprising of fat, protein, moisture and carbohydrates and are not perfectly stable (two samples of the same product are always different) (Burns & Ciurczak, 2001; Prieto *et al*., 2017). Including a wide range of chemical variability (different batches of sardine samples) as well as recording samples over a period of time to incorporate as much spectral data in the calibration could probably have increased the prediction accuracy (Burns & Ciurczak, 2001). The accuracy of the predicted constituents is not only affected by sample size but by a sum of different errors such as sampling errors, instrumental errors, reference method errors and regression model errors (Williams & Norris, 1987; Burns & Ciurczak, 2001). The aforementioned errors make it difficult to obtain an RPD value that is ≥ 3 (Burns & Ciurczak, 2001). Another contributing factor is the measurement of muscle foods in the transition between frozen and thawed products, which

according to Fornés and Chaussidon (1978) serverely influence NIR signal and water interaction (Tøgersen *et al*., 2003).

Prediction models could be improved with a large data set (>100) that includes a large spectral variation from wavelengths above 1100nm. This NIR region covers spectral data that is important for predicting chemical components (Prieto *et al*., 2003). Measuring the samples under the same condition, with the same sample presentation and uniform temperature could provide more improved and reliable prediction models. In this study, reference methods were affected mostly by low variability in the data set and difficulties in simultaneously conducting reference analysis of the sardine samples. These constraints hindered the prediction of fish quality using NIR spectroscopy, considering the fact that fish is a highly perishable product that requires stable temperature control to avoid the transition between freezing and thawing.

Owing to its simple, non-destructive and fast characteristics, the NIR method is already used extensively in the food, agriculture and pharmaceutical industries. In conclusion, although the NIR analysis could not predict the proximate and fatty acid composition of sardines in this study, the NIR method can undoubtfully be used as an analytical technique in both scientific and industrial level. However, in order to obtain prediction models that are accurate enough to be used in quality and process control, a larger data set that includes a large variability should be used with more components and other multivariate regression methods such as principal component regression (PCR) and compare the results with PLSR. In revealing the internal structure (morphophysiological indicators) of whole fish scanned on the skin and homogenised sample set, it is recommended that a classification method such as soft independent modelling of class analogy (SIMCA) or partial least squares discriminant analysis (PLS-DA) be investigated.

References

Burns, D.A. & Ciurczak, E.W. (2001). Handbook of Near-Infrared Analysis, 2nd Edition. New York: Marcel & Dekker.

Coetzee, J.C., Van der Lingen, C.D., Hutchings, L. & Fairweather, T.P. (2008). Has the fishery contributed to a major shift in the distribution of South African sardine? *ICES Journal of Marine Science*, **65**, 1676-1688.

Cozzolino, D., Murrya I., Chree, A., Scaife, J.R. (2005). Multivariate determination of free fatty acids and moisture in fish oils by partial least squares regression and near-infrared spectroscopy. *LWT*, **38**, 821-828.

De Goede, J. (2004). Spatial and temporal variability in lipid content and gonadosomatic index of sardine (*Sardinops sagax*) in Southern Benguela ecosystem. Master of Technology Thesis. Cape Technikon. South Africa.

Feng, Y., Ma, L., Du, Y., Fan, S. & Dai, R. (2012). Chemical composition of three important fish species (sardine, anchovy, and mackerel). *Advanced Material Research*, **554-556**, 900-904.

Fornés, V., & Chaussidon, J. (1978). An interpretation of the evolution with temperature of the V_2+V_3 combination band in water. The Journal of Chemical Physics, **68**(10), 4667-4671.

Huang, H., Liu, L. & Ngadi, M. O. (2014). Recent Developments in Hyperspectral Imaging for Assessment of Food Quality and Safety. *Sensors,* **14**, 7248-7276.

Hale. M. 1984. Proximate chemical composition and fatty acid composition of three coastal pelagic fishes. *Marine Fisheries Review*, **46**(1): 19-21.

Patterson, K.R. (1992). An improved method of studying the condition of fish with an example using Pacific sardine *Sardinops sagax* (Jenys). *Journal of Fish Biology*, **40**, 821-831.

Prieto, N., Pawluczyk, O., Russell Dugan, M.E. & Aalhus, J.L. (2017). A review of the principles and applications of near infrared spectroscopy to characterize meat, fat and meat products. *Applied Spectroscopy*, **71**(1), 403-1426.

Saeed, M., Ali Reza, A., Hamid, F., Maryan, S. & Nuria, P. (2012). Determination of protein and moisture in fishmeal by near-Infrared reflectance spectroscopy and multivariate regression based on partial least squares. *Iran Journal of Chemical Engineering,* **31**(3). 51-59.

Suseno, S.H., Syari, C., Zakiyah, E.R., Jacoeb, A.M., Izaki, A.F., Hayati, S. (2014). Chemical composition and fatty acid profile of small pelagic (*Amblygaster sirm* and *Sardinella gibbosa*) from Muara Angke, Indonesia. *Oriental Journal of Chemistry*, **30**(3), 1153-1158.

Tøgersen, G. Arnesen, J.F., Nilsen, B.N. & Hildrum, K.I. (2003). On-line prediction of chemical composition of semi-frozen ground beef by non-invasive NIR spectroscopy. *Meat Science*, **63**, 515-523.

Van der Lingen, C.D. & Hutchings, L. (2005). Estimating the lipid content of pelagic fish in the southern Benguela by visual assessment of their mesenteric fat. *African Journal of Marine Science*, **27**(1), 45-53.

Williams, P.C. & Norris, K. (1987). *Near Infrared Technology in the Agricultural and Food Industries*, 2nd Edition. American Association of Cereal Chemist: St Paul, MN.