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Chemical and near-infrared determination of moisture, fat and protein in tuna fishes

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Abstract

Moisture, protein, free fat and total fat were determined in Tuna Fishes (38) skipjack (*Katsuwonus pelamis*) (20) and yellow fin (*Thunnus albacares*) (18) by chemical methods. Moisture was determined by freeze-drying or lyophilization, oven-dry methods and by using electronic moisture analyzer. Protein content was determined using Gerhardt semi-micro Kjeldahl and combustion methods. Total fat was determined using acid-hydrolysis method and free fat was determined by Soxhlet method. Near-infrared spectra of the fishes (28) and partial least square regression with the reference methods namely lyophilization method for moisture, combustion method for proteins, acid-hydrolysis method for total fat and Soxhlet method for free fat were used to set a calibration model. This regression model was then used for quantifying the named components of the fishes (5), considered as unknowns, from their near-infrared spectra. There is a good comparison between the results from the different chemical methods and the components quantified using the near-infrared spectroscopy method. An outcome of this work is that near-infrared spectroscopy can serve as an accurate and fast method for quantifying the components of fishes.

Keywords: Near-infrared spectroscopy; Moisture; Fat; Protein; Fish; Partial least squares regression

1. Introduction

Industrialization has led to an increase in the consumption of pre-processed foods and thus the concern of food components and its quality has grown (Ritter, 2005). Wet chemical analysis has been used to characterize and quantify the components of foodstuffs but the main disadvantages are that the procedures are costly, destructive and labour intensive (Clark & Short, 1994). There has been an increasing use of spectroscopy (Baeten & Dardenne, 2002; Belton, 1997; Jhaumeer-Laulloo & Ramasami, 2005), in particular near-infrared spectroscopy (NIR) (Cozzolino & Murray, 2002; Goula & Adamopoulos, 2003; Jhaumeer-Laulloo & Ramasami, 2005; Kays, Archibald, & Sohn, 2005; Kempe & Luchetta, 2003; Kurowski, Timm, Grummisch, Meyhack, & Grunewald, 1998; Laporte & Paquin, 1999; Lee, Cavinato, Mayes, & Rasco, 1992; Priego-Capote, Ruiz-Jiménez, García-Olmo, & Luque de Castro, 2004; Rannou & Downey, 1997; Takamura, Endo, & Matoba, 1998; Wilson & Tapp, 1999), for quantitative analysis of food with advances in hardware and chemometrics. The main near-infrared spectroscopy advantages are its rapid and non-destructive characteristics (Baeten & Dardenne, 2002; Osborne, 2000). NIR (Hart, Norris, & Golumbic, 1962) is more advantageous compared to mid-infrared spectroscopy (MIR) for the quantitative analysis of food. In fact in NIR there is a low degree of absorbance due to the low degree of excitation of harmonics and combination vibrations. Thus the sample for transmission measurement can be a few mm thick. Fur-

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ther there is a high degree of reflected light and therefore measurement in diffuse reflectance is also possible.

NIR has been commonly used for the routine analysis of fish flesh composition (Cozzolino, Murray, & Scaife, 2002; Downey, 1996; Huang et al., 2003; Lin, Cavinato, Huang, & Rasco, 2003; Nortvedt, Torrissen, & Tuene, 1998; Solberg & Fredriksen, 2001; Vogt, Gormley, Downey, & Somers, 2002). Downey (1996) used NIR spectroscopy for the determination of oil and moisture in farmed salmon carcasses. Different sample sites (294) comprising of six selected sites on the dorsal and ventral surfaces on each side of the fish were investigated. The mean oil and moisture contents were of 7.5 and 69.7%, respectively, for dorsal calibration, and 12.4% and 66.3% for ventral calibration. The standard errors reported for oil and moisture contents were 2.0% and 1.45%, respectively, for dorsal calibration, and 2.4 and 1.9% for ventral calibration. Nortvedt et al. (1998) used NIR transmittance spectroscopy to determine the amount of fat, protein and dry matter in wet homogenized Atlantic halibut (*Hippoglossus hippoglossus*) fillet. Vogt et al. (2002) compared the accuracy of four methods for the determination of fat content in fresh herring (*Clupea harengus*). The methods used were the Torry Fatmeter, a microwave method, near-infrared reflectance spectroscopy and a commercial modification of the Soxhlet procedure. The standard errors for fat by these methods were 1.37%, 0.79%, 0.80% and 1.36%, respectively. Huang et al. (2003) used short-wavelength NIR reflectance spectrometer (600–1100 nm) to determine moisture and salt in cured Atlantic salmon (*Salmo salar*). The results obtained using partial least square regression (PLS) calibration gave salt and moisture with corresponding ranges of 0.13–12.3% w/w and 49.7–74.2%, respectively. The root mean square errors for these predictions were 1.37% w/w for salt and 2.04% w/w for water. Lin et al. (2003) used short-wavelength NIR spectrometer (600–1100 nm) to determine salt content in chinook (*Oncorhynchus tshawytscha*) and chum salmon (*O. Keta*). The total salt content ranged from 1.66% to 5.95% w/w (SEP 0.32% w/w) for chinook and 2.15% to 5.69% w/w (SEP 0.25% w/w) for chum salmon while the moisture ranged from 50.7% to 71.6% w/w for Chinook and 55.5% to 69.7% w/w for chum salmon.

In continuation with our work using infrared spectroscopy (Jhaumeer-Laulloo, Rondeau, Cadet, & Ramasami, 2003; Ramasami, Jhaumeer-Laulloo, Cadet, Rondeau, & Soophul, 2005; Ramasami et al., 2004) and analysis of food components (Jhaumeer-Laulloo & Ramasami, 2004), the aim of the present study is to explore the use of NIR spectroscopy for quantifying moisture, total and free fat and protein in thirty eight tuna fishes namely twenty skipjack (*Katsuwonus pelamis*) and eighteen yellow fin (*Thunnus albacares*) obtained from "Tuna Fishing and Canning Industries Limited" which were fished in the mauritian territorial seas. These fishes, from other sources, have been the targets for various studies (Cabello et al., 2003; Shimamoto, Hiratsuka, Hasegawa, Sato, & Kawano, 2003). With this purpose, classical analysis using reference methods in combination with near-infrared spectra were used to set the calibration models. These models were used for the quantification of the named components in tuna fishes.

According to the literature, bands have been assigned in near-infrared region and these are helpful for identifications. Absorptions bands for water, proteins and lipids can be observed at different wavelengths and these have been reported (Lee et al., 1992; Osborne, Fearn, & Hindle, 1993). The peaks attributed to water are 760 nm, 1450 nm and 1940 nm while those attributed to proteins are 1510 nm, 1980 nm, 2050 nm and 2180 nm. In NIR reflectance spectroscopy C–H vibration can occur throughout the spectrum. According to previous reports (Downey, 1996; Miller & Hongis, 1989; Murray & Williams, 1990), absorbance bands in the 1100–1390 nm region are largely attributed to the C–H stretching mode and overtone. Lipids also show peaks at 1722 nm, 1760 nm, 2310 nm and 2346 nm (McClure & Stanfield, 2002).

2. Material and methods

2.1. Samples and sample preparation

Skipjack ($n = 20$) and yellow fin ($n = 18$) samples were kept frozen until both chemical and NIR spectroscopy analysis were performed. Samples were cut and thawed before chemical analyses and NIR measurements were performed.

2.2. NIR measurement

NIR spectra were recorded on an Analytical Spectral Device (ASD) LabSpec Pro NIR analyzer using a fibre optic probe (Bifurcated Fibreoptic Reflectance Probe). Samples were scanned at 25 different sites with an increment of 2 nm between 350 and 2500 nm with a fast scanning time of 0.1 s. Spectra were collected randomly all over fish surface without skin. The spectra were exported for computation as JCAMP files. Each spectrum was assigned the corresponding concentration value obtained by the reference method.

2.3. Reference analysis

The reference methods used for the different components are validated methods currently used for quality control. As they are destructive methods they were performed on the fish sample after NIR measurement. All determinations were performed in triplicate.

2.4. Moisture content

Moisture content was determined using three different methods namely freeze-drying or lyophilization (Seligman & Farber, 1971), oven-dry (AOAC 950.46, 1990) and electronic moisture analyzer SCALTEC SMO 01 (Gottingen, Germany). Uncertainty of the reference determination was expressed as the standard deviation.

2.5. Fat content

The fat content of the sample was determined as free fat and the total fat (EEC 90/496, 1990). Free fat was extracted from lyophilized sample by Soxhlet (ISO 1444, 1996) using ether as solvent. The total fat content was determined by the acid-hydrolysis method (AOAC 948.15, 1995). Minced samples (1.5 g) were digested with dilute hydrochloric acid (5 ml) for about 45 minutes on a water bath. The mixture obtained was then extracted with a combination of solvents comprising of methanol (2.5 ml), diethyl ether (7.5 ml) and petroleum ether (7.5 ml). Thereafter the mixture was centrifuged, ether–fat layer was decanted and evaporated and the fat content was measured.

2.6. Protein content

Lyophilized sample was used to estimate the protein content. Protein content was estimated from nitrogen (N × 6.25) using Gerhardt semi-micro Kjeldahl method (AOAC 979.09, 1990) and the Leco CHNS 932 apparatus based on combustion method (AOAC 990.03, 1990).

2.7. Data analysis

Near IR spectra were exported from the Analytical Spectral Device (ASD) in JCAMP format for chemometric analysis in The Unscrumbler software (version 7.1, CAMO ASA, Norway). Calibration models between chemical data and NIR spectra were developed using PLS2 regressions with full cross validation. The principle of partial least squares regression (PLS) has been described in detail in several papers (Cadet & Guardia, 2000; Rondeau, Sers, Jhurry, & Cadet, 2002). In order to avoid overfitting of the models, the optimum number of terms in the PLS calibration models was determined as indicated by the lowest number of factors that gave the closest to minimum value of the prediction residual error sum of squares (PRESS) function in cross validation (Naes, Isaksson, Fearn, & Davis, 2002).

3. Results and discussion

3.1. Moisture content

Moisture contents for the three chemical methods are reported in Table 1. Analysis of the moisture contents for the fishes indicate that the amount obtained from the oven and freeze methods are comparable while that obtained from moisture analyzer is systematically lower.

3.2. Fat content

Values for crude free fat content measured by Soxhlet method are in the range 0.25–1.69% (w/w) for *K. pelamis* with standard deviation ranging from 0.01% to 0.10%. The free fat content for *T. albacares* is in the range of 0.50–3.20% (w/w) with standard deviation ranging from 0.14% to 0.49%. Total fat content determined by the acid hydrolysis method is in the range 3.47–5.73% (w/w) for *K. pelamis* and 3.10–5.70% (w/w) for *T. albacares* with standard deviation ranging from 0.03% to 0.99% and from 0.14% to 0.28%, respectively.

3.3. Protein content

The Kjeldahl method is the most common reference method for the determination of protein in fish. The crude protein content determined by the Kjeldahl method is in the range 21.80–25.10% for *T. albacares* with standard deviation ranging from 0.35% to 1.13% and 20.72–27.98% for *K. pelamis* with standard deviation ranging from 0.01% to 0.10%. However this method is quite tedious and time consuming as it involves three main steps namely digestion, distillation and titration. Moreover during these processes lot of waste is produced which is not environment friendly.

The other reference method for the determination of protein is the combustion method. This method is faster and it requires only small amount of sample. The crude protein content determined by the combustion method is in the range 21.00–26.00% for *T. albacares* with standard deviation ranging from 0.28% to 0.85% and 19.02% to 27.90% for *K. pelamis* with standard deviation ranging from 0.01% to 0.07%. These results indicate a good correlation between the protein content obtained from the two methods.

3.4. Spectra

The twenty-five raw spectra of each sample were averaged. Figs. 1(a) and (b) show the averaged spectrum of skipjack in the regions 900–1800 nm and 1800–2300 nm, respectively. For water, the band at 967 nm arises from the overtone combination band of the O–H stretch and

Table 1
Moisture content using reference methods

	Katsuwonus pelamis			*Thunnus albacares*		
	Oven dry (%)	Freeze drier (%)	Moisture analyzer (%)	Oven dry (%)	Freeze drier (%)	Moisture analyzer (%)
Range	66.5–72.3	69.7–76.6	63.5–67.1	70.3–72.7	71.3–73.8	60.6–63.4
MinSD	0.01	0.04	0.03	0.14	0.14	0.02
MaxSD	0.66	1.07	1.02	0.78	0.71	0.92

MinSD, minimum standard deviation; MaxSD, maximum standard deviation.

bending mode, while the peaks at 1435 nm and 1940 nm correspond to the first overtone of O–H stretch and combination bands of O–H. Absorbance peaks related to fatty acids are largely attributed to the C–H and CH_2 vibrations. The first overtone of C–H stretch is in the range 1718–1760 nm and the second overtone of the C–H stretch lies in the range 1100–1390 nm. The prominent band at 928 nm can be assigned to the overtone C–H of the methylene group in lipids. Several bands are also apparent for protein (1550, 2055, 2180 nm).

3.5. *Mathematical analysis*

Mathematical analysis is highly important in near-infrared spectroscopy. This is because near-infrared spectra generally consist overlapping vibrational bands that may appear non-specific and poorly resolved. Chemometrics is used to sort out these spectroscopic limitations. All statistical calculations were carried out with the commercial analysis "Unscrumbler" software. Different calibration models such as principal component analysis (PCA), prin-

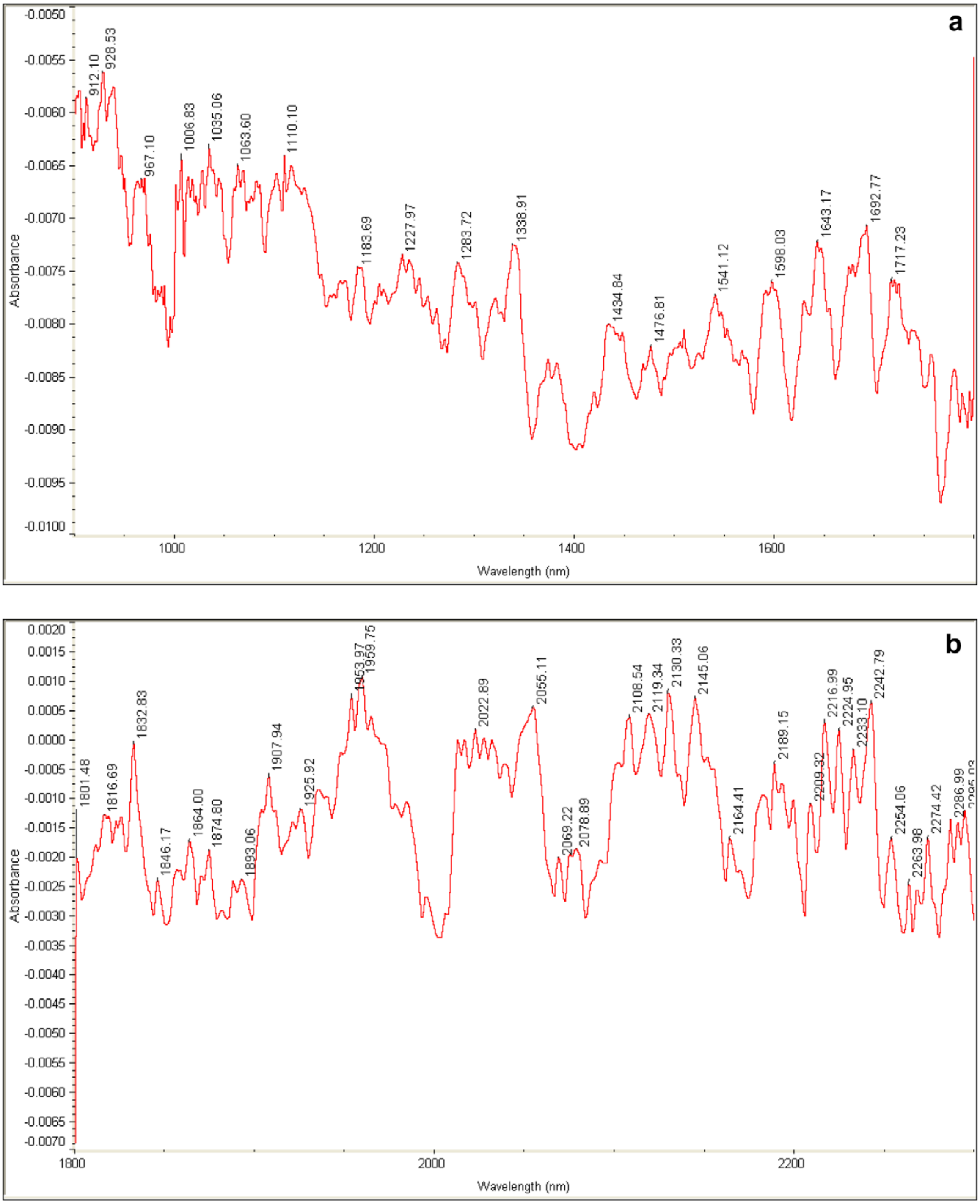

Fig. 1. Average spectrum of skipjack in the region 900–1800 nm (a) and 1800–2300 nm (b).

Table 2
Prediction results of the different components (%) by NIR method after PLS calibration using reference methods and cross validation

Fish	Moisture			Proteins			Total fats			Free fats		
	Lyophilization method	NIR method	Deviation	Combustion method	NIR method	Deviation	Hydrolysis method	NIR method	Deviation	Soxhlet method	NIR method	Deviation
1	72.0	71.8	0.2	24.00	24.16	−0.16	3.10	2.99	0.11	2.00	1.94	0.06
2	70.9	70.8	0.1	24.40	24.36	0.04	5.00	5.05	−0.05	3.20	3.16	0.04
3	71.7	71.6	0.1	23.50	23.44	0.06	4.80	4.85	−0.05	2.20	2.21	−0.01
4	71.0	71.1	−0.1	24.70	24.73	−0.03	4.20	4.24	−0.04	2.90	2.60	0.30
5	72.7	72.6	0.1	22.70	22.62	0.08	3.40	3.50	−0.10	2.20	2.22	−0.02
11	70.6	71.1	−0.5	23.60	23.66	−0.05	5.20	5.06	0.14	2.10	2.32	−0.22
12	70.3	70.1	0.2	25.00	24.72	0.28	5.70	5.78	−0.08	2.00	1.80	0.20
14	70.3	70.3	0.0	26.00	26.15	−0.15	5.20	5.00	0.20	0.80	0.81	−0.01
15	71.7	71.7	0.0	24.30	24.64	−0.34	4.90	4.72	0.18	1.00	1.45	−0.45
16	70.5	70.6	−0.1	24.70	24.91	−0.21	4.60	4.58	0.02	0.60	0.52	0.08
19	70.9	71.0	−0.1	21.30	21.04	0.26	4.00	3.92	0.08	1.80	1.92	−0.12
21	69.9	69.7	0.2	24.80	24.69	0.11	4.60	4.64	−0.04	0.25	0.22	0.03
22	71.8	71.9	−0.1	23.90	23.63	0.27	4.81	4.78	0.03	0.50	0.51	−0.01
23	67.4	67.7	−0.3	27.90	27.70	0.20	3.47	3.21	0.26	1.04	1.18	−0.14
24	69.9	69.7	0.2	27.30	26.98	0.32	4.51	4.36	0.15	1.23	1.15	0.08
25	70.1	69.9	0.2	25.97	26.30	−0.33	4.30	4.63	−0.33	0.68	0.66	0.02
27	72.3	72.2	0.1	24.64	24.56	0.08	3.95	3.99	−0.04	0.84	0.83	0.01
28	70.1	70.1	−0.1	24.61	24.34	0.27	4.62	4.76	-0.14	1.09	1.01	0.08
29	70.4	70.6	−0.2	25.19	25.57	−0.38	4.41	4.41	0.00	0.94	1.11	−0.17
30	70.4	70.4	0.0	20.52	20.55	−0.03	5.39	5.25	0.15	0.77	0.74	0.03
31	66.5	66.3	0.2	21.82	22.03	−0.21	5.63	5.62	0.01	0.87	0.97	−0.10
32	70.6	70.8	−0.2	22.61	22.39	0.22	5.73	5.51	0.22	0.23	0.22	0.01
33	70.3	70.5	−0.2	21.22	21.51	−0.29	4.56	4.63	−0.07	1.69	1.68	0.01
36	69.7	69.7	0.0	23.44	23.31	0.13	3.86	3.86	0.00	0.73	0.69	0.04
37	71.7	71.6	0.1	21.76	21.79	−0.03	3.56	3.59	−0.03	0.68	0.53	0.15
38	69.4	69.4	0.0	24.75	24.60	0.15	4.24	4.58	−0.34	1.47	1.25	0.22
39	70.4	70.3	0.1	22.66	22.68	−0.02	4.43	4.40	0.04	0.59	0.57	0.02
40	70.3	70.3	0.0	23.69	23.91	−0.22	3.88	4.17	−0.29	0.79	0.90	−0.11

cipal component regression (PCR), multi linear regression (MLR) could have been used. In fact, partial least square regression (PLS-2) was used, since it calibrates for all components simultaneously.

Partial least square (PLS-2) was performed on selected calibration set of 28 average spectra of *T. albacares* and *K. pelamis* and applied in the region 600–2500 nm (with 1701 data points used as principal variables). Twenty-seven axes were calculated for the regression model and ten axes were considered to construct linear models for each variable. The reference methods used for moisture and proteins are the combustion and lyophilization methods, respectively. The predicted amount of moisture, protein, total and free fat from NIR and reference methods together with the prediction error are given in Table 2. The correlation coefficient (R^2) of the prediction values against reference values of the construction models were used as indicators of the quality of the construction models. The plots for the different components yielded straight lines with R^2 being 0.98, 0.99, 0.95 and 0.96 for moisture, protein, total fat and free fat, respectively. These values show that the different components correlated highly for the samples in this study. The regression model was used for quantification of five fishes samples, not included into the calibration, considered as unknown. The results are reported in Table 3. Regression analysis for the different components and the NIR predictor for the five unknowns are not as good as for the twenty-eight fishes considered as standards and this may be attributed to the small size for the fishes considered as unknowns.

Table 3
(%) comparison by chemical and NIR methods for the five fishes considered as unknowns

Fish	Moisture			Proteins			Total fats			Free fats		
	Lyophilization method	NIR method	Deviation	Combustion method	NIR method	Deviation	Hydrolysis method	NIR method	Deviation	Soxhlet method	NIR method	Deviation
		Predicted			Predicted			Predicted			Predicted	
10	71.5	70.4	1.2	24.70	23.58	1.12	4.10	4.41	−0.31	3.00	2.22	0.78
18	72.0	69.9	2.1	23.20	22.69	0.51	4.60	4.88	−0.28	0.50	0.80	−0.30
26	70.3	70.5	−0.2	27.40	24.28	3.12	4.22	3.45	0.78	0.76	1.55	−0.79
34	70.7	71.4	−0.7	19.02	26.73	−7.71	4.36	4.01	0.35	1.12	0.79	0.33
35	69.6	71.3	−1.7	23.75	22.01	1.74	4.23	5.72	−1.49	0.84	1.92	−1.08

4. Conclusions

To summarise, chemical methods and near-infrared spectroscopy have been used for the prediction or moisture, protein, free fat and total fat in Tuna fishes (*K. pelamis*) and yellow fin (*T. albacares*) from the mauritian territorial seas. The proximal compositions of moisture, fat and protein for these two species for the calibration model from the different methods have good correlations. These fishes (Cabello et al., 2003) from the pacific and atlantic oceans have been analysed by AOAC methods (AOAC, 1994). These quantifications are in agreement with the findings of the current study. Although similar correlations are poorer for the unknowns, possibly due to small sample size, NIR spectroscopy has the potential for rapid, accurate and non-destructive determination of the different components in fishes.

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