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The effects of freeze-drying and storage on the FT-Raman spectra of Atlantic mackerel (*Scomber scombrus*) and horse mackerel (*Trachurus trachurus*)

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Abstract

FT-Raman spectroscopy was undertaken for quantitative characterization of lipids in horse mackerel (*Trachurus Trachurus*) and Atlantic mackerel (*Scomber scombrus*). Peroxide value of fish lipids and protein extractability of the fish lipids and muscles were also measured by titration and Bradford assay, respectively.

Raman spectral analysis of oil extracted from freeze-dried Atlantic and stored (12 weeks) mackerel and horse mackerel revealed significant reductions in the intensity of bands associated with CH_2 stretches and C=O ester stretches. This was also confirmed by an increase in the intensity of the bands at 3011 cm^{-1} and 2960–2850 cm^{-1}, suggesting alterations in lipid structure involving CH groups. An initial rise followed by a decline in peroxide value of the oil extracted from freeze-dried mackerel and horse mackerel stored at 22 °C for 12 weeks confirmed the occurrence of lipid oxidation. Structural changes in the proteins of freeze-dried mackerel and freeze-dried horse mackerel stored for 12 weeks at 22 °C resulted in a decrease in the solubility of myofibrillar proteins in salt solution.

Keywords: Atlantic mackerel; Horse mackerel; Freeze-drying; Lipids; Proteins; FT-Raman spectroscopy

1. Introduction

Raman spectroscopy is primarily used in biochemistry to investigate protein structures and recently its use has become popular in determining the functions and interactions of proteins in a food matrix (Howell, Herman, & Li-Chan, 2001; Li-Chan, 1996; Nai-Teng, 1977). As the cells and tissues used in Raman spectroscopy can be intact and as no homogenisation is required prior to analysis of the samples, accurate information can be obtained from Raman spectroscopy with regard to the concentration, structure and interaction of the biochemical molecules (Marquardt & Wold, 2004).

Many studies using Raman spectroscopy have been reported on proteins, including protein–protein interactions, which occur during processing and storage of many food products (Howell, 1992; Marquardt & Wold, 2004). Some studies have also been conducted to elucidate protein–lipid interactions in emulsions (Howell et al., 2001). Raman spectroscopy has also been used to investigate the structure of lipids and changes resulting from storage conditions or lipid oxidation (Howell et al., 2001). Fatty acids in lipids and their degree of saturation can influence the intensity and location of bands in the spectrum of the lipids. Raman spectroscopy provides information on the vibrational motions of molecules, which are measured in terms of the shift in frequency or wavenumber. It is a non-destructive method that can be used in different ways, including monitoring changes in lipid C–H stretching vibrations, which are normally seen within the 2800–3000 cm^{-1} region (Li-Chan, 1996).

Studies on fatty acids include those carried out on oleic acid, investigating polymorphism or interaction isomerisation superimposed on chain–chain interactions (Devlin & Levin, 1990; Howell, 1994; Verma & Wallach, 1977). Using

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Raman spectroscopy any changes in various regions of the lipid structure such as CH stretching, C=O stretching, CH_2 scissoring and C–C stretching caused by oxidation or interaction with other structures such as proteins can be measured (Tandon, Forster, Neubert, & Wartewig, 2000). Raman spectroscopy can also provide accurate quantitative measurement of total degree of unsaturation of lipids, as indicated by changes in the ratios of C=C stretching band near 1660 cm^{-1} to the C=O stretching band near 1750 cm^{-1} or CH_2 scissoring band near 1445 cm^{-1} (Sadeghi-Jorabchi, Hendra, Wilson, & Belton, 1990; Sadeghi-Jorabchi, Wilson, Belton, Edwads-Webb, & Coxon, 1991). Levels of saturation can be detected at the 3013, 1663 and 1264 cm^{-1} and the ratio of *cis* and *trans* isomers and their conjugated products as a result of oxidation can also be detected using several bands in the regions of 1655 and 1670 cm^{-1}, respectively (Agbenyega, Claybourn, & Ellis, 1991).

Raman spectroscopy has been used to investigate the structural changes of fatty acids from corn oil (Howell et al., 2001), oleic acid (Devlin & Levin, 1990; Verma & Wallach, 1977) or protein–protein and lipid–protein interactions in emulsions (Aslanian, Negrerie, & Chambert, 1986; Badii & Howell, 2002; Ngarize, Herman, Adams, & Howell, 2003; Yager & Gaber, 1987). Many of the studies have placed significant emphasis on changes in the lipid components of mostly biological membranes or other fatty acids, for example, from vegetable oils. Our previous

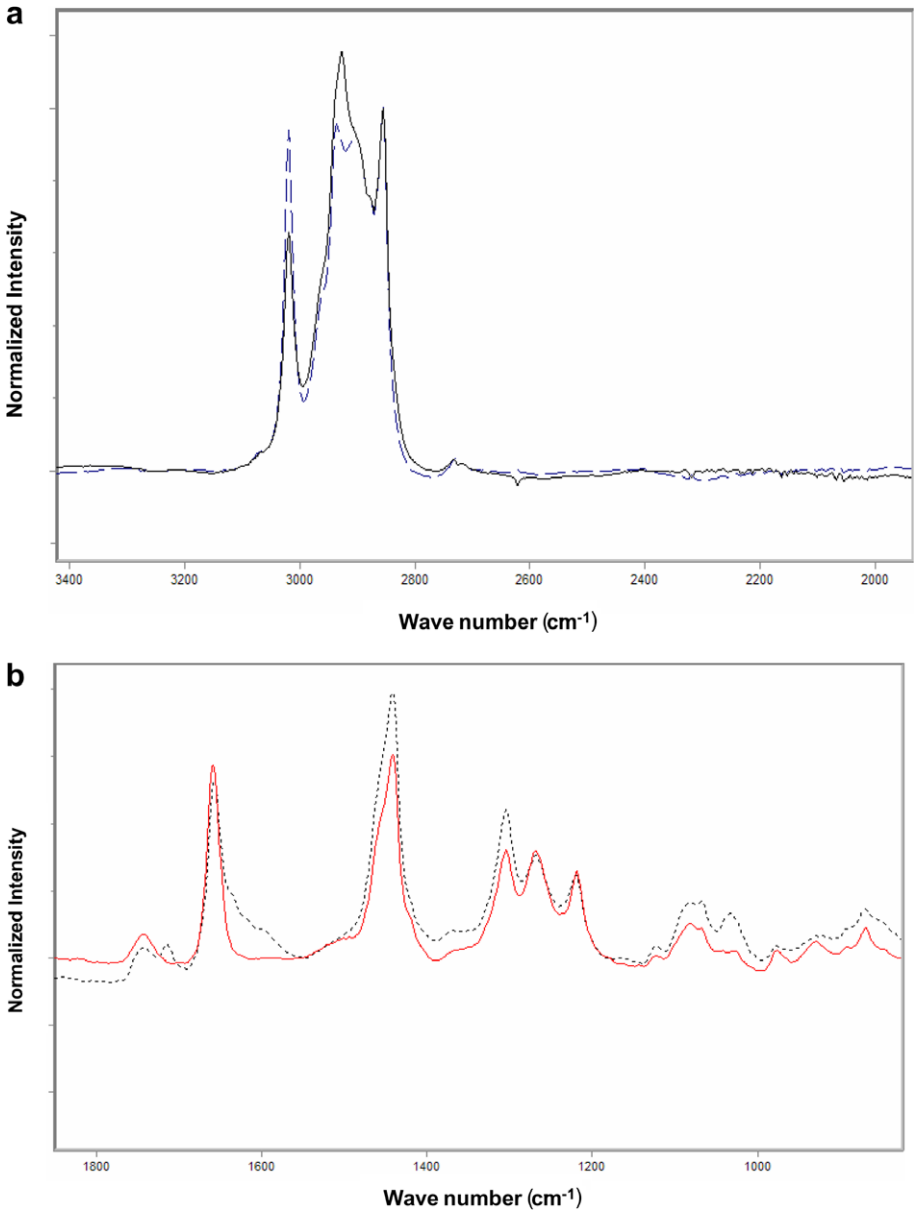

Fig. 1. FT-Raman spectra of freeze-dried and fresh Atlantic mackerel. The oil spectra are from (a) 2000–3500 cm^{-1} and (b) 800–1800 cm^{-1} for oil extracted from freeze-dried mackerel (dashed line) and oil extracted from fresh mackerel (solid line).

studies have focused primarily on changes in the protein components of fish muscle and not on the lipid components (Badii & Howell, 2002). In the previous studies it was shown that lipid oxidation causes alteration in proteins extracted from fish as a result of cell damage by ice crystal formation in the muscle during frozen storage. This subsequently results in a reduction in protein solubility and formation of aggregates (Badii, Zhdan, & Howell, 2004; Saeed & Howell, 2004). As proteins contribute to the texture of food, such changes subsequently affect the rheological properties, such as texture and eating quality of badly stored frozen fish, leading to a loss of a healthy protein source (Badii & Howell, 2002).

Polyunsaturated fatty acids (PUFA) are easily oxidised during cooking and frozen storage, *via* a series of chain reactions that eventually lead to changes in flavour, texture and aroma of food, collectively known as organoleptic changes, and rancidity. Freezing is a very useful method for delaying oxidation and inhibiting microbial deterioration of fish muscle. However, inappropriate storage of frozen fish clearly results in rapid denaturation of myosin followed by actin and changes in the flavour and texture of the flesh (Bligh & Dyer, 1959; Bradford, 1976). Another method which is considered to be effective in delaying spoilage is drying. There is a wide range of drying processes; the most common ones are freeze-drying, air-drying and sun drying. The latter two are currently used in developing countries as they provide a cheaper alternative for transporting products.

Changes in the structure of lipids (extracted from both mackerel and horse mackerel) were assessed using FT-Raman spectroscopy. Oxidation levels were also monitored by measuring the peroxide values. The effect of freeze-drying on the myofibrillar proteins was also assessed, by measuring solubility changes during storage at 22 °C for 12 weeks.

2. Materials and methods

2.1. Materials

Deboned fillets of Atlantic mackerel (*Scomber scombrus*) and horse mackerel (*Tmchurus tmchurus*) were supplied by M & J Seafood, Farnham, UK. Sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate were bought from BDH/Merck Chemicals Ltd., A Lutterworth, UK. All reagents used were analytical grade.

2.2. Methods

2.2.1. Sample preparation

Atlantic mackerel and horse mackerel fillets were stored at −80 °C overnight prior to freeze-drying and storage at 22 °C. Oil was extracted from the freeze-dried fish using Bligh and Dyer method (1959). The oil as well as the fish muscle proteins were separately analysed by FT-Raman spectroscopy, together with control samples of non-freeze-dried mackerel and horse mackerel.

2.2.2. Peroxide value

The chloroformic layer (5 ml) obtained during lipid extraction was evaporated off under oxygen-free nitrogen gas. Once fully evaporated, chloroform (10 ml) was added to the oil and washed into the flask, followed by acetic acid (15 ml). Potassium iodide (1 ml) was added, the flask was closed and swirled to mix the solution and placed in a dark cupboard. Milli-Q water (75 ml) was added after 1 min and 1 ml of starch solution in water (1%) was added as an indicator. The mixture was titrated with diluted 0.0025 M sodium thiosulfate solution. A control flask without the food oil was run alongside the sample. The peroxide value (PV) was calculated as follows:

Table 1
Relative peak intensity of the Raman bands in the region 800–3200 cm^{-1} for oil immediately extracted from freeze-dried Atlantic mackerel and fresh Atlantic mackerel, respectively

Peak assignment (wavenumber ±2 cm^{-1})[a]	Relative peak intensity	
	Fresh	Freeze-dried
Symmetric CCC stretch various CCC stretches 869 (874)	0.049 ± 0.005	ND
Symmetric CCC stretch various CCC stretches 925 (916)	0.038 ± 0.007	ND[b]
Symmetric CCC stretch various CCC stretches 966 (973)	0.031 ± 0.008	ND
Anti-symmetric CCC 1078 (1083)	0.059 ± 0.006	0.088 ± 0.004
1215	0.135 ± 0.004	0.135 ± 0.005
=C–H symmetric rock (*cis*) (1265)	0.169 ± 0.003	0.168 ± 0.004
CH_2 phase twist (1303)	0.170 ± 0.004	0.234 ± 0.02
CH_2 scissoring (1441)	0.271 ± 0.02	0.424 ± 0.02
C=C stretch (*cis*) (1660)	0.291 ± 0.01	0.268 ± 0.003
C=O ester stretch 1745 (1750)	0.039 ± 0.005	0.029 ± 0.002
CH_2 symmetric stretch (2855)	1 ± 0.0	1 ± 0.0
CH_2 anti-symmetric stretch 2928 (2933)	0.951 ± 0.05	1.173 ± 0.03
=CH stretch of =CHR or $=CH_2$ groups (3011)	0.938 ± 0.02	0.695 ± 0.06

[a] The spectra were an average of 64 scans which were baseline corrected and normalized to the intensity of 2855 cm^{-1}. Each sample was done in triplicates and the standard deviation is shown next to the peak intensities of the samples. The values in parenthesis next to the peak assignments are the values cited by Howell et al. (2001).

[b] ND = not detected.

$$\mathrm{PV} = \frac{1000(a-b)c}{w} \quad (1)$$

with a and b the amounts of sodium thiosulfate solution used in the sample and the blank (ml), respectively, c the concentration of thiosulfate solution, and w the amount of sample used (g).

2.2.3. Protein determination by the Bradford method

Protein solutions were prepared by diluting a stock bovine serum albumin (BSA) standard solution (Badii & Howell, 2002) in sodium chloride solution. The protein standard (0.1 ml) was pipetted into a test tube, using the diluent as a blank. Coomassie protein assay reagent (3 ml) was added to each test tube an mixed well by vortexing. The absorbance was read at 595 nm within 90 min. Protein concentration (c_{protein}) was calculated as follows:

$$c_{\mathrm{protein}} = \frac{c_{\mathrm{conc}} V_{\mathrm{sup}}}{w} \quad (2)$$

with c_{conc} the concentration of undiluted protein solution (mg ml^{-1}) and V_{sup} the supernatant volume (ml).

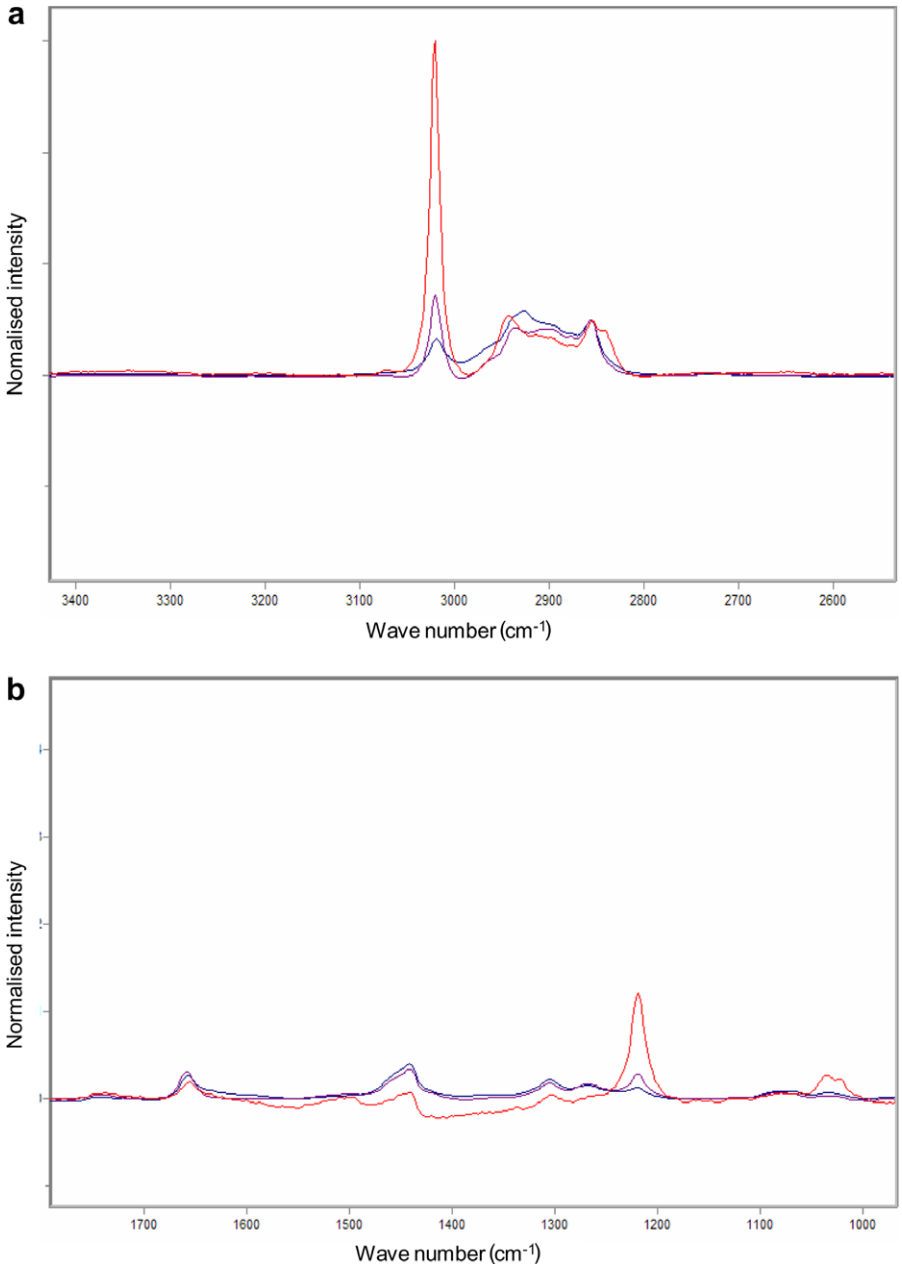

Fig. 2. FT-Raman spectra of freeze-dried Atlantic mackerel. The oil spectra are from (a) 2500–3400 cm^{-1} and (b) 900–1800 cm^{-1} for oil extracted from freeze-dried mackerel stored for 2 weeks (blue line) 4 weeks (purple line) and 12 weeks (red line) at 22 °C (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

2.2.4. Raman spectral analysis

The Raman scattering of samples placed in 7 ml glass containers (FBG-Anchor, London, UK) was measured at ambient temperature on a Perkin–Elmer (Wellesly, MA) System 2000 FT Raman spectrophotometer with excitation from an Nd:YAG laser at 1064 nm. Frequency calibration of the instrument was performed using the sulfur line at 217 cm^{-1}. The analysis was carried out in triplicate. The recorded spectrum was analysed using Grams 32 software (Galactic Industries Corp., Salem, NH). The oil spectra collected were baselined, and the intensity was normalised on 2855 cm^{-1} for oil (Marquardt & Wold, 2004). Assignments of the bands in the spectra to protein or A oil vibrational modes were made on the basis of the literature (Agbenyega et al., 1991; Aslanian et al., 1986; Howell et al., 2001; Marquardt & Wold, 2004; Sadeghi-Jorabchi et al., 1991; Yager & Gaber, 1987).

3. Statistical analysis

For peroxide value and protein solubility measurements, triplicate samples were used and the mean values as well as the standard deviations are reported. In the Raman spectroscopy for oil extracted from fresh and freeze-dried mackerel and horse mackerel, three replicates were tested.

Raman spectra of oil extracted from fresh mackerel and freeze-dried mackerel were compared to each other using the unpaired t-test with Welch's correction (not assuming equal variances). Raman spectra of oil from freeze-dried fish stored for different times were compared to each other using a one-way ANOVA (Kruskal–Wallis statistics) test, where a Gaussian distribution was not assumed. A *post hoc* test was applied in order to identify which groups were significantly different from each other. All the statistical analyses were carried out using the SPSS (Chicago, IL) package.

4. Results and discussion

4.1. Analysis of the oil extracted from freeze-dried and fresh Atlantic mackerel

Fig. 1(a) shows the spectra in the 2000–3500 cm^{-1} region of the oils extracted from freeze-dried and fresh mackerel in. Both spectra indicated bands assigned to the following groups: CH_2 symmetric stretch at 2855 cm^{-1}, CH_2 anti-symmetric stretch at 2928 or 2936 cm^{-1} in the oil spectrum of freeze-dried and fresh mackerel, respectively, and =CH stretch of =CHR or $=CH_2$ groups at 3011 cm^{-1}. The intensity of the band at 2936 cm^{-1} and shoulder at 2902 cm^{-1} in the oil spectrum of fresh mackerel was lower than those detected in the oil spectrum of freeze-dried mackerel ($P \leqslant 0.05$). However, the band at 3011 cm^{-1} was higher in the spectrum of oil extracted from fresh mackerel.

Fig. 1(b) shows the spectra of oils extracted from freeze-dried and fresh mackerel in the 800–1800 cm^{-1} region. The oil spectrum indicated bands assigned to the following groups: C=O ester stretch at 1745 cm^{-1}, C=C stretch at 1659 cm^{-1}, CH_2 scissoring at 1441 cm^{-1}, CH_2 in phase twist at 1304 cm^{-1}, =C–H sym rock (*cis*) at 1267 cm^{-1}, anti-symmetric CCC stretch at 1083 cm^{-1} with a shoulder at 1067 cm^{-1}, and various CCC stretches at 976 cm^{-1}, 870 cm^{-1} with a shoulder at 891 cm^{-1} (Howell et al., 2001). The intensity of the bands in spectra of oil extracted from freeze-dried and fresh mackerel were similar with the exception of the following bands: 1442, 1304, 1123, 1082, 1031 and 870 cm^{-1}, the intensities of which were much higher in the oil spectrum of freeze-dried mackerel (Table 1) ($P \leqslant 0.05$).

Table 2
Relative peak intensity of the Raman bands in the region 1000–3200 cm^{-1} for oils extracted from freeze-dried mackerel and freeze-dried horse mackerel stored at 22 °C for 2, 4 and 12 weeks

Perk assignment (wave number)[a]\ Storage time (weeks)	Relatively peak intensity					
	Mackerel			Horse mackerel		
	2	4	12	2	4	12
Anti-symmetric CCC stretches 1075 (1083)	0.077 ± 0.004	0.087 ± 0.004	ND[b]	0.096 ± 0.002	0.073 ± 0.002	NB
1221	0.253 ± 0.02	0.124 ± 0.009	1.186 ± 0.008	0.314 ± 0.02	0.134 ± 0.001	1.201 ± 0.006
=C–H symmetric rock (*cis*) (1265)	0.170 ± 0.005	0.157 ± 0.004	ND	0.134 ± 0.003	0.166 ± 0.003	ND
CH_2 Phase twist (1303)	0.167 ± 0.003	0.235 ± 0.02	0.074 ± 0.004	0.203 ± 0.003	0.162 ± 0.002	0.055 ± 0.002
CH_2 Scissoring (1441)	0.351 ± 0.009	0.372 ± 0.04	0.079 ± 0.003	0.415 ± 0.02	0.312 ± 0.02	0.080 ± 0.003
C=C stretch (*cis*) (1660)	0.325 ± 0.02	0.268 ± 0.03	0.291 ± 0.06	0.266 ± 0.03	0.309 ± 0.02	0.199 ± 0.001
C=O ester stretch 1745 (1750)	0.06 ± 0.005	0.0286 ± 0.003	0.062 ± 0.006	0.033 ± 0.003	0.043 ± 0.004	0.058 ± 0.001
CH_2 symmetric stretch (2855)	1.00 ± 0.0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.013
CH_2 anti-symmetric stretch (2933)	0.865 ± 0.03	1.136 ± 0.02	1.10 ± 0.03	0.994 ± 0.05	0.818 ± 0.04	1.709 ± 0.04
=CH stretch of =CHR or $=CH_2$ groups (3011)	1.451 ± 0.02	0.650 ± 0.02	6.061 ± 0.04	0.653 ± 0.02	1.439 ± 0.08	8.024 ± 0.07

[a] The spectra were an average of 64 scans which were baseline corrected and normalized to the intensity at 2855 cm^{-1}. Each sample was done in triplicate; standard deviation is shown next to the peak intensities of the samples. The values in Parenthesis next to the peak assignment are the values in the reference by Howell et al. (2001).

[b] ND = not detected.

5. Analysis of oil spectra of stored and non-stored freeze-dried mackerel

The spectra of oil extracted from freeze-dried mackerel stored at 22 °C for 2, 4 and 12 weeks are shown in Fig. 2(a) and (b). The oil spectrum indicated bands assigned to the following groups: anti-symmetric CCC stretches at 1083 cm^{-1}, =C–H symmetric rock (*cis*) at 1265 cm^{-1}, CH_2 phase twist at 1303 cm^{-1}, CH_2 scissoring at 1441 cm^{-1}, C=C stretch (*cis*) at 1660 cm^{-1}, C=O ester stretch at 1750 cm^{-1}, CH_2 symmetric stretch at 2855 cm^{-1}, CH_2 anti-symmetric stretch at 2933 cm^{-1} and =CH stretch of =CHR or CH_2 groups at 3011 cm^{-1}. The differences in the intensity of the bands in oil spectra of freeze-dried mackerel at different time points were statistically significant ($P \leqslant 0.05$) (Table 2).

A significant decrease was detected in the intensity of the above bands in the oil spectrum of freeze-dried mackerel at 12 weeks compared to the oil spectra at 2 and 4 weeks of storage at 22 °C ($P \leqslant 0.05$); except for the band at 1221 cm^{-1}, where the intensity was higher at 12 weeks compared to 2 or 4 weeks ($P \leqslant 0.05$). The intensity of the bands at 1660 and 1750 cm^{-1} was lower ($P \leqslant 0.05$) in the oil spectrum of stored (4 weeks) than stored (2 weeks) freeze-dried mackerel. In both the spectra of oil extracted from stored (2 and 4 weeks) freeze-dried mackerel a band assigned to 2933 cm^{-1} (CH_2 anti-symmetric stretch) was detected which shifted to 2927 and 2939 cm^{-1} in 2 weeks and 4 weeks, respectively. The intensity of this band was significantly lower in the oil spectrum of stored (4 weeks) freeze-dried mackerel ($P \leqslant 0.05$); in this spectrum, 2 additional bands were detected at the intensity of 2905

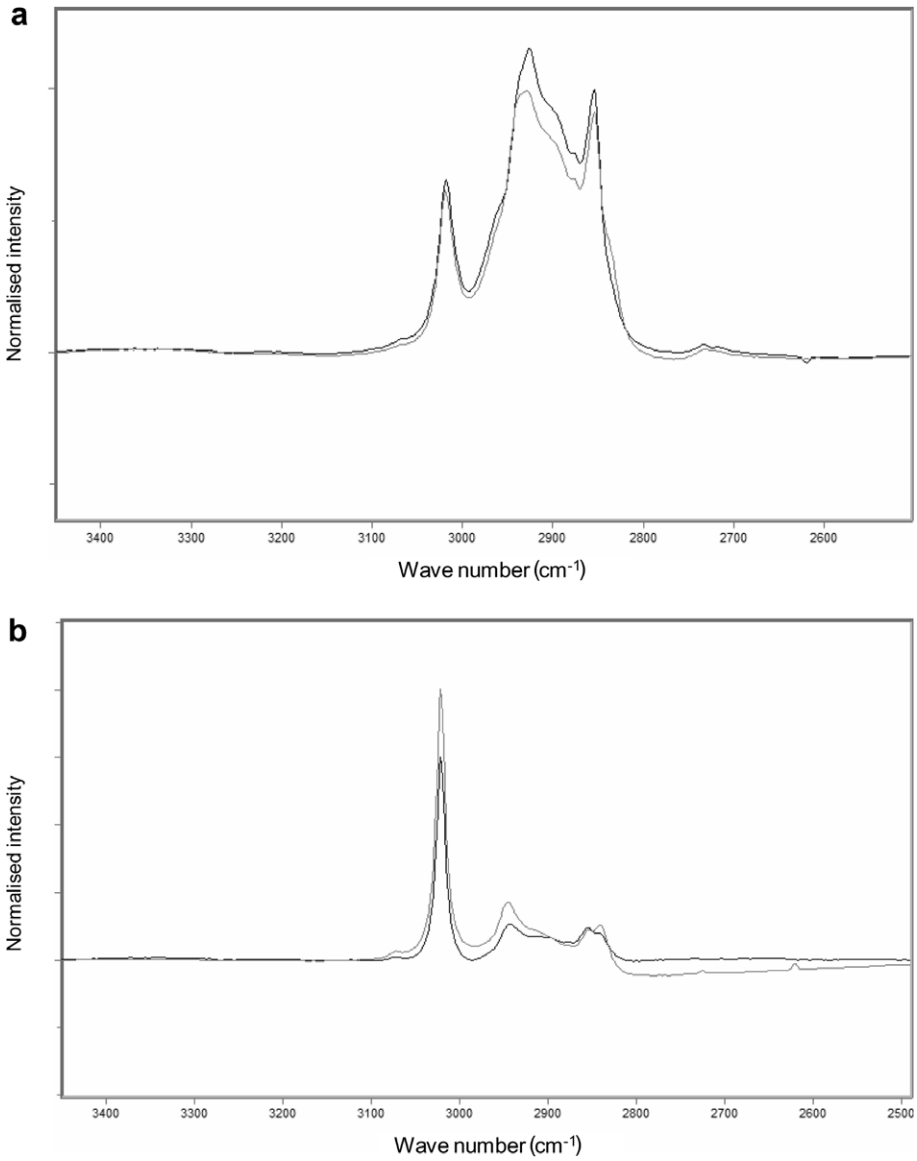

Fig. 3. FT-Raman spectra of freeze-dried Atlantic mackerel and horse mackerel. The spectra are from 2500 to 3400 cm^{-1} for oil extracted from freeze-dried mackerel (blue line) and horse mackerel (red line) stored for (a) 2 weeks and (b) 12 weeks (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

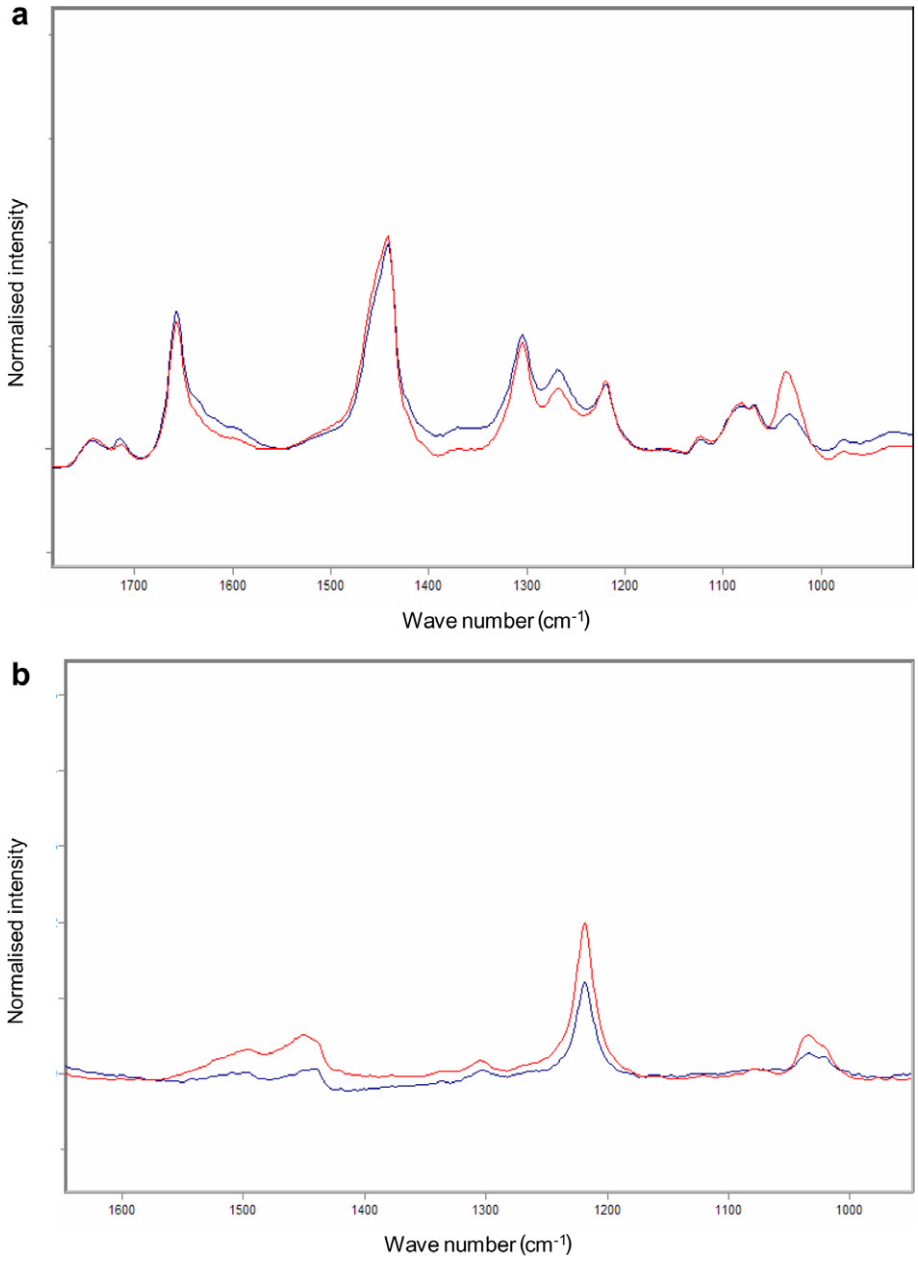

Fig. 4. FT-Raman spectra from 900 to 1700 cm^{-1} for oil extracted from freeze-dried Atlantic mackerel (blue line) and horse mackerel (red line) stored for (a) 2 weeks and (b) 12 weeks (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

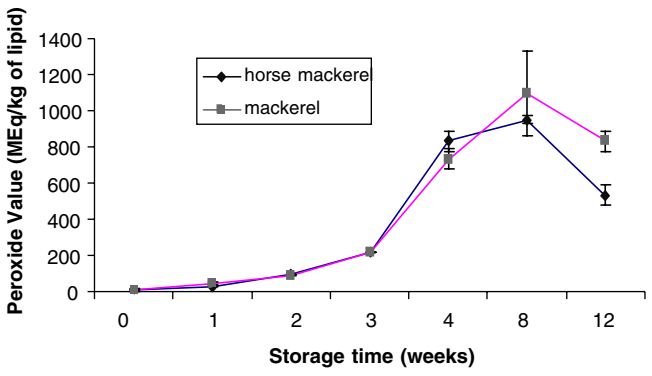

Fig. 5. Peroxide value obtained from freeze-dried Atlantic mackerel and horse mackerel stored at 22 °C for 12 weeks.

(assigned to R_3C–H, CH_3 symmetric or CH_2 anti-symmetric stretch) and 1715 cm^{-1} (Howell et al., 2001).

The intensity ratios of the peaks at I_{1660}/I_{1750},I_{1660}/I_{1445} and $I_{1265}/_{1303}$ were measured in the Raman spectra of oil extracted from freeze-dried mackerel. The ratios were significantly different in oil samples of freeze-dried mackerel stored for 2, 4 and 12 weeks. The ratio intensity of the band I_{1660}/I_{1750} in oil extracted from freeze-dried mackerel decreased in the following order: 2 > 4 > 12 weeks (36.27, 10.13 and 2.88), respectively. The ratio intensity of I_{1660}/I_{1445} in oil extracted from freeze-dried mackerel decreased in the following order: 2 > 4 > 12 weeks (2.04, 0.87 and 0.58), respectively. The ratio intensities of I_{1660}/I_{1750} and I_{1660}/I_{1445} determine the total unsaturated fatty acids; this

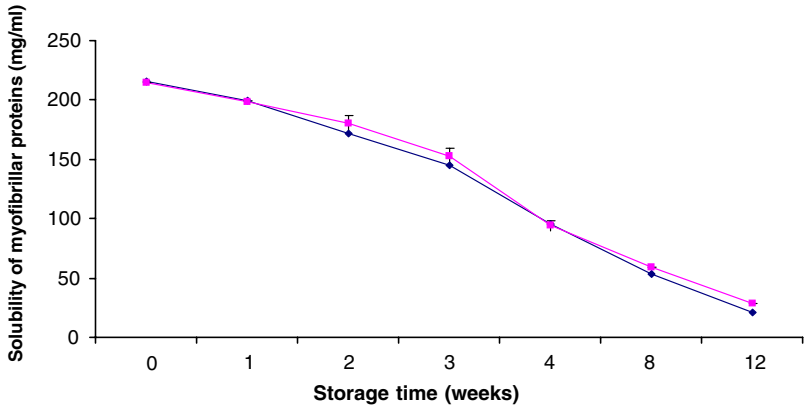

Fig. 6. Protein extractability results of freeze-dried Atlantic mackerel and horse mackerel stored at 22 °C for 12 weeks.

means that the total number of unsaturated fatty acids decreased with increasing storage at 22 °C. The ratio intensity of I_{1265}/I_{1303} was higher in the oil Raman spectra of freeze-dried mackerel stored for 4 weeks than both 2 and 12 weeks (0.950, 0.659 and 0.776 cm^{-1}, respectively). This ratio intensity is used for rapid determination of the total *cis* isomer in the oil. This means that the total *cis* isomer in the oil from freeze-dried mackerel decreased with increasing time of storage at 22 °C.

6. Comparison of analysis of the oil spectra of stored freeze-dried horse mackerel and stored freeze-dried mackerel

Changes observed in the intensity of the bands detected in the oil spectra of freeze-dried horse mackerel were very similar to those detected in the oil spectra of freeze-dried mackerel at different time points. In the oil spectrum of freeze-dried horse mackerel at 12 weeks, similar to that of freeze-dried mackerel, the intensity of the bands decreased significantly ($P \leqslant 0.05$) compared to the oil spectra of freeze-dried horse mackerel stored for 2 and 4 weeks at 22 °C (Figs. 3 and 4 and Table 2).

7. Analysis of the peroxide value

This test showed an increase in lipid oxidation products with storage time (Fig. 5). The peroxide value increased gradually up to 3 weeks. After 4 weeks peroxide value continued to rise at a slower rate until 8 weeks, when the value decreased sharply. There were no significant differences in the peroxide value between freeze-dried mackerel and freeze-dried horse mackerel up to 4 weeks of storage ($P \geqslant 0.05$). However, the peroxide value of freeze-dried mackerel increased to a higher level after 8 weeks of storage at 22 °C, compared to that of the freeze-dried horse mackerel kept under the same conditions ($P \leqslant 0.01$). The major difference between the peroxide value of freeze-dried mackerel and freeze-dried horse mackerel, measured at 12 weeks after storage at 22 °C, supports the significant differences between the oil spectra of freeze-dried mackerel and horse mackerel after storage.

8. Analysis of protein extractability

The initial contents of salt-soluble proteins were 210 mg g^{-1} for both freeze-dried horse mackerel and mackerel fillets. These values decreased gradually during the 12 weeks of storage at 22 °C (Fig. 6). There were no significant differences in the protein extractability between mackerel and horse mackerel ($P \geqslant 0.05$). However, a significant reduction was seen in the protein extractability in both mackerel and horse mackerel from the start of the storage time until the end of the storage at 12 weeks ($P \leqslant 0.001$). After 4 weeks at 22 °C, protein extractability in NaCl solution was reduced by approximately 50% for both mackerel and horse mackerel. After 12 weeks at 22 °C, protein extractability was reduced by about 80% in both mackerel and horse mackerel. These changes indicated alterations in the structure of fish proteins during processing and storage.

9. Conclusion

Freeze-drying affects the structure and composition of fish lipids, as shown in the oil spectra of freeze-dried mackerel and horse mackerel obtained using Raman spectroscopy. Such changes are particularly prominent during the long term storage of freeze-dried fish at 22 °C. An increase in the region corresponding to 2960–2850 cm^{-1} in the oil spectra of both freeze-dried mackerel and horse mackerel was detected, which suggests alterations in lipid structure involving CH groups and hydrophobic interactions. Lipid structural changes, as indicated by the intensity of the bands, were more obvious in freeze-dried mackerel oil, compared with freeze-dried horse mackerel. This is possibly due to a higher percentage of eicosapentaenoic acid (EPA) in mackerel (6.23%) than in horse mackerel (2.02%), as shown by earlier studies on fatty acid composition of mackerel and horse mackerel (unpublished data, Sarkardei and Howell, 2005); the higher PUFA content of mackerel makes it more susceptible to structural changes due to lipid oxidation, compared with horse mackerel. This was confirmed by the formation of primary oxidation products (peroxide value), which were higher in mackerel

than horse mackerel ($P \leqslant 0.01$). Lipid oxidation also affects the solubility and extractability of fish myofibrillar proteins. This is caused as a result of changes in the secondary structure of fish muscle proteins due to the formation of intramolecular cross-linkages, as well as hydrophobic interactions. These interactions reduce the myofibrillar protein extractability in freeze-dried fish samples. This has been previously reported in frozen fish (Badii & Howell, 2002; Badii et al., 2004). Dried fish suffers from many defects and, generally, the process of drying with or without salt causes severe damage to proteins and lipids and to the organoleptic properties. Nevertheless, poor communities throughout the world are reliant on dried products and steps should be taken to improve their quality with for example, antioxidants.

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