Indian Journal of Pure & Applied Physics
Vol. 55, July 2017, pp.525-531

Fourier self deconvolution and principle components analysis of spirulina based food products in quality assessment

S Karthikeyan[a]* & K Vizhiselvi[b,c]

[a]Department of Physics, Dr Ambedkar Government Arts College, Chennai 600 039, Tamil Nadu, India

[b]Department of Physics, J N N Institute of Technology, Chennai 601 012, Tamil Nadu, India

[c]Research and Development Centre, Bharathiyar University, Coimbatore 641 046, Tamil Nadu, India

Received 25 May 2016; revised 23 January 2017; accepted 27 February 2017

Biochemical constituent's change in the spirulina based substance items have been studied utilizing Fourier transform infrared spectroscopy (FTIR). The FTIR spectra demonstrate the changes in band area, and intensity which have been used to find both qualitative and quantitative changes in carbohydrate, lipid and protein in the given sample. The quantitative changes in biochemical composition have been studied by measuring the intensity ratio of the chosen band I_{2924}/I_{2853}, I_{2925}/I_{1615}, I_{1545}/I_{1657} and I_{2960}/I_{2873}. The lipid/protein ratio (I_{2925}/I_{1615}) increments from the chosen commercial samples examined recommending spirulina have regular antioxidative agents. The band area ratio of the methyl, methylene group ($\upsilon sCH_3/\upsilon asCH_2+\upsilon sCH_2$) has been computed for a change in fatty acids compositions. Fourier self deconvolution has been utilized to study the biochemical composition changes in commercial samples. The increases in the secondary structure of proteins such as α helix, β turn and β sheet have been seen among the sample. Further the changes in the band area of glycogen, lactic acid have been seen in the spectra after deconvolution. The utilization of principle components analysis (PCA) helps us to find a distinct variation in the sample. PCA demonstrates the prominent band appeared to be highly influenced is amide I and glucose. It has been found that FTIR with PCA can be utilized as a part of subjective and quantitative examination of spirulina based food products.

Keywords: FTIR, Spirulina, Food products, PCA, Proteins, Carbohydrates, Glycogen, Deconvolution

1 Introduction

Spirulina is unicellular and filamentous blue, green algae of high protein (70%), minerals, vitamins, essential fatty acids and carbohydrates. It is regularly found in the aquatic environment like lakes and rivers. It assumes a critical part of human and animal nutrition, natural security through waste water reusing and energy conservation. It can be grown in both in fresh water and waste water. Spirulina is a photo autotrophic filamentous cynanobacterium with cylindrical shaped multi cell trichomes in an open left hand helicoidal shape[1]. Besides the antioxidant properties, plantensis has pulled in the considerations of analysts. The spirulina has been attributed as a perfect nourishment and dietary supplement for the 21st century by the FAO of the unified nations[2]. The verification of nourishment fixings is of pivotal worry to sustenance processors since the immaculateness of sustenance fixings is effectively subject to mishandling by corrupt suppliers[3,4]. Further, such sustenance item that is being defiled because of expansion of fixings will hurt customers. Spirulina is one such nourishment item that is liable to examination for subjective and quantitative investigation. Checking nourishment quality, well being has turned into the attention on customer and safety administrative concern. Spirulina assumes an essential part that is subjected to qualitative as well as quantitative analysis.

The utilization of FTIR spectroscopy in the algal biomass examination has been helpful in observing biochemical changes[5-7]. Fourier transforms infrared spectroscopy gives effectively checking varieties in characteristic groups and vibrations. It can give information about the peak, intensity, width and wave number region. FTIR allows with more noticeable affectability to describe the nature of organic substance and its compositional esteem. Further the utilization of chemometrics in combination with FTIR spectra has been valuable for the separation of biological samples. The spectra taken from the sample was utilized to build multivariate calibration and to decide the quantitative distinction between the diverse samples. PCA is likely the most widespread multivariate statistical method used as a part of chemometrics and it is viewed by

*Corresponding author (E-mail: physicskarthik@gmail.com).

numerous as the procedure that is most important in the context of data examination. It is a direct non parametric methodology for isolating appropriate information from confusing data sets. Principle component analysis is utilized for data reduction to identify a small number of factors in a large number of variables. The PCA replaces the contribution to scores and loadings which are the qualities of primary segments. The scores plot permits the perception of grouping among samples. It allows an essential assessment in the middle of classification similarity and is exceptionally valuable for visual review of complex information contexts. The loadings plots were utilized to figure out which spectral region most contributed to the difference in the dataset. Fundamental favorable positions of this strategy are to extract the quantitative information about large information sets. In this paper, biochemical changes in spirulina and its commercial products have been examined utilizing FTIR in combination with PCA.

2 Materials and Methods

2.1 Sample collection

The commercial sample of food products is acquired from neighborhood markets with extreme safety measures to guarantee that sample is produced using spirulina rich raw products. The samples were numbered FS1, FC1, FP1, FA1, and immaculate spirulina test was developed in laboratory conditions and named as raw samples. The samples oven dried at 40 °C in a hot air oven for 60 min. At that point test was powdered with agate mortar. The powdered sample was utilized for further investigation.

2.2 FTIR analysis

The powdered sample is blended with Kbr and recorded with the Perkin Elmer spectrum FTIR spectrometer accessible with SAIF at IIT, Chennai in the absorption mode of 4000-400 cm^{-1}. For every sample, 200 scans were averaged with a spectral resolution of 4 cm^{-1}. The spectrometer was persistently purged with dry nitrogen. Every sample of equivalent weight was brought with the extreme care by applying weight and pellets of uniform size. Three replicates of every sample were taken and the average spectrum was recorded. The recorded spectrum was further utilized for investigation with Origin 8.0.

2.3 Principle component analysis

The Principle component analysis (PCA) was performed with SPSS 16.0 programming. It was mainly utilized for data reduction to identify a small number of factors that explain the majority of the change observed in a much larger variable. The absorbance value of samples of three distinct region fatty acids (3050-2800 cm^{-1}), proteins (1800-800 cm^{-1}) and carbohydrates (1200-900 cm^{-1}) is given as input for the 5 samples as $n \times n$ matrix and the factor reduction obtained by principle component analysis. The input absorption value of the sample is formed as (540×5) data matrices that transform the original measurement variables into new uncorrelated variables called principal components. It transforms the inputs into scores and loadings which are the characteristics of principal components. It is utilized for quantitative strategies discriminating samples. The scores of the component were plotted to gather data responsible for variability in the FTIR information. Each one of the outcomes was expressed as mean ± standard deviation. The student's t test was performed to discover the differences between control and the commercial sample. The p values less than 0.05 were considered statistically significant.

3 Results and Discussion

3.1 Intensity ratio of the FTIR spectra

Figure 1 demonstrates the average FTIR spectra of raw and commercial sample in 4000- 400 cm^{-1} region. Tentative frequency assignment is displayed in Table 1. Since the infrared spectra of spirulina are made out of a few groups of various functional groups, for example, proteins, lipids and polysaccharides point by point detailed investigation was performed in the region 3600-3200 cm^{-1}, 3050-2800 cm^{-1} and 1800-800 cm^{-1}. The relative concentrations of the functional group belonging to various macromolecules were determined by measuring the area under the related absorption

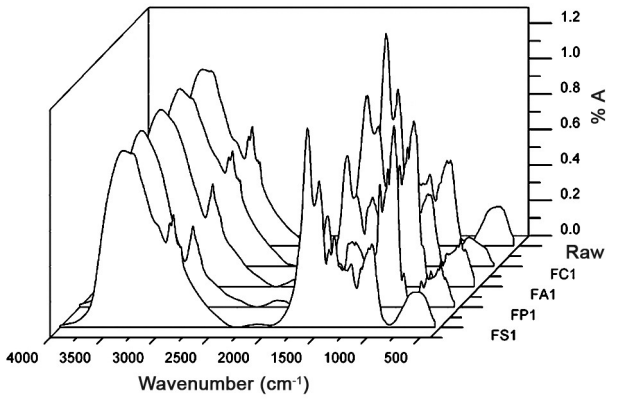

Fig. 1 — Average FTIR spectra of spirulina and its commercial products in the region of 4000-400 cm^{-1}

Table 1 — Tentative frequency assignment and their functional group for raw and commercial sample of spirulina

Assignments	Functional group	Raw	FS1	FC1	FP1	FA1
OH and NH stretching	Saccharides and proteins	3416(vs)	3406(vs)	3426(vs)	3416(vs)	3406(vs)
Proteins Proteins Proteins Proteins						
Amide A	Proteins	3294(vs)	3297(vs)	33301	3276	3294(s)
CH_3 assym stretching	Lipid	2958(m)	2960(m)	2959(m)	2969(m)	2960(s)
CH_2 assym stretching	Lipid	2925(m)	2927(m)	2925(m)	2927(m)	2926(s)
CH_3 sym stretching	Proteins	2873(m)	2873(m)	2872(m)	2870(m)	2872(m)
CH_2 bending	Lipids and proteins	2854(m)	2852(m)	2853(m)	2852(m)	2854(m)
Amide I (C=O)	Protein	1650(vs)	1660(vs)	1650(s)	1659(s)	1650(s)
Amide II (N-H,C-N)	Protein	1538(s)	1545(s)	1565(s)	1534(s)	1532(s)
Methyl (CH_3) out of plane	Protein, lipids, saccharides	1450(w)	1450(m)	1460(m)	1450(m)	1454(s)
COO symmetric stretching	Fatty acids	1401(w)	1410(m)	1397(m)	1400(m)	1402(m)
C-O,C-C,C-O-C stretching	Saccharides	1147(w)	1128(w)	1142(w)	1132(m)	1126(s)
C-O stretching	Glycogen	1075(w)	1079(w)	1074(w)	1073(m)	1075(s)
Pyranose ring vibrations	Saccharides	1024(w)	1019(w)	1014(w)	1021(w)	1015(s)

*vs-very strong, s-strong, m-medium, w-weak, vw-very weak

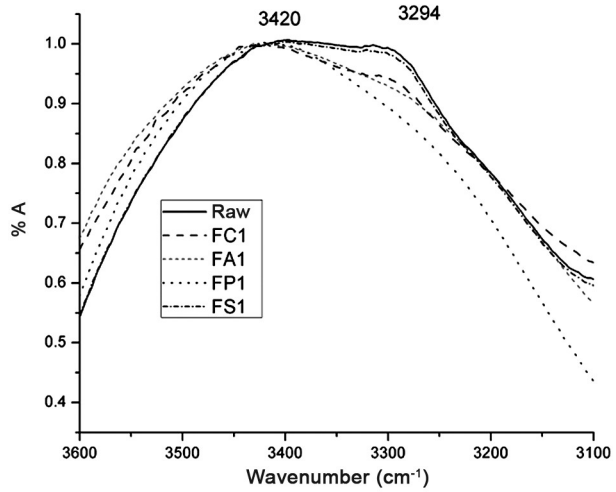

Fig. 2 — Average spectra normalized at amide A in the region 3600-3100 cm^{-1}

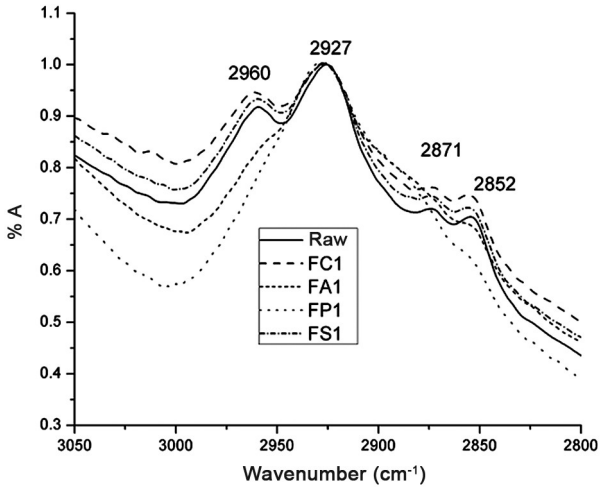

Fig. 3 — Average spectra normalized with respect to CH_2 asymmetric stretching band in the region of 3050- 2800 cm^{-1}

bands[8-10]. Figure 2 demonstrates the normalized band of 3420 cm^{-1} in the region of 3600-3100 cm^{-1}. As seen in Fig. 2, a wide band observed at ~3418 cm^{-1} is assigned to hydrogen bonded intermolecular OH group and shoulder at 3294 cm^{-1} is assigned to the N-H stretching[11]. For all the sample shifts to higher frequency as for raw were seen with the exception of FP1 (Table 2). Likewise from Fig. 2 the band area of amide A increments in the sample FA1, FP1 and FC1. For the sample FS1 it diminishes when compared to the raw sample.

Figure 3 shows the normalized spectra at ~2927 cm^{-1} in the region 3050-2800 cm^{-1}. The CH_2 asymmetric (~2927) and symmetric (~2852) are assigned to lipids[12-14]. As seen from Fig. 3 the band area associated with lipids (~2930 cm^{-1} & 2854 cm^{-1}) and fatty acids (~2958 cm^{-1}) increase in all commercial samples when compared to a raw sample of spirulina. This may be due to the use of antioxidants as a food additive which enables the protection for spirulina based food products on undesirable oxidative changes. In addition spirulina contains natural sources of tocopherols, ascorbic acid as natural antioxidants. This may prevent the oxidations of lipids during food processing[15]. Table 2 shows the intensity ratio of the selected band obtained from the FTIR spectra. From Table 2 the ratio of lipid/protein (I_{2925}/I_{1615}) increases from all commercial samples except for FA1 & FP1 suggesting spirulina has natural antioxidative agents which prevent lipids per oxidations thereby increasing the level of lipids during food processing. This antioxidant will inhibit the generation free radicals such as aldehydes, ketones and

epoxids and active oxygen species which present the reaction of lipid-protein cross links, thereby capable of maintaining the quantity of lipids[16]. In addition to the changes in molecular level of lipids were calculated from the ratio of specific bands and it is reported in Table 2. This variation in the ratio of absorption of the methyl and the methylene group ($\upsilon sCH_3/\upsilon asCH_2+\upsilon sCH_2$) indicates the presence of a long chain of fatty acids. It is observed from Table 2 that a low value of this ratio of the sample FA1 & FP1 indicates long chain fatty acids. Further the ratio of $\upsilon asCH_2/\upsilon sCH_2$ (I_{2924}/I_{2853}) can be used in monitoring the state of biological membranes[17]. A high value of the ratio is observed in the commercial sample FP1, FC1 indicates lower disorder and a lower motion freedom in a lipid cycle chain when compared to other samples.

According to Ozaki *et al.*[18], the ratio of peak intensities of the bands of 1550 cm^{-1} and 3300 cm^{-1} (I_{1550}/I_{3300}) could be used as an indicator of the relative concentration of protein to water included near the surface of biological tissues. It is found that this ratio decreases in all commercial samples, suggesting decreasing from protein contents with respect to the raw sample. Also an increase or a decrease in the ratio of the intensities of the amide bands at 1545 cm^{-1} and 1657 cm^{-1} could be attributed to a change in the composition of the whole protein pattern[19-20]. Further the ratio of the intensity of absorption of methyl and methylene bands could be used as the main contribution of the number of methyl groups of protein fibers. The intensity ratios of I_{1545}/I_{1657} and I_{2960}/I_{2873} decrease indicating decrease in protein contents in all the commercial samples.

3.2 Fourier self deconvolution in the analysis of proteins, lipids and carbohydrates

The infrared spectra of spirulina are made out of various functional groups, for example, proteins, lipids and polysaccharides. Hence detailed investigation was performed in the region 3600-3200 cm^{-1}, 3050-2800 cm^{-1} and 1800-800 cm^{-1}. The relative concentrations of the functional group belonging to various macromolecules were determined by measuring the area under the related absorption bands[8-10]. Figure 2 demonstrates the normalized band of 3420 cm^{-1} in the region of 3600-3100 cm^{-1}. As observed from Fig. 2 wide band observed at ~3418 cm^{-1} is assigned to hydrogen bonded intermolecular OH group and shoulder at 3294 cm^{-1} is assigned to the N-H stretching[11]. For all the sample shifts to higher frequency as for raw were seen with the exception of FP1 (Table 2). Likewise from Fig. 2 the band area of amide A increments in the sample FA1, FP1 and FA1. For the sample FS1 it diminishes when compared to the raw sample.

The utilization of the second derivative and curve fitting investigation is exceptionally regular to study the secondary structure of protein in amide I region. The amide bands of proteins are susceptible to changes in secondary structure and are the most regularly utilized as a part of vibrational investigation. These vibrations include protein folding with hydrogen bonding with peptide bonds. From Fig. 4, the peaks at 1659 cm^{-1} were assigned to α helix, 1672 cm^{-1} assigned to β sheet, 1644 cm^{-1} to a random coil and 1674 cm^{-1} to β turns. The % band area of the secondary structure of protein in the amide I band is exhibited in Table 3.

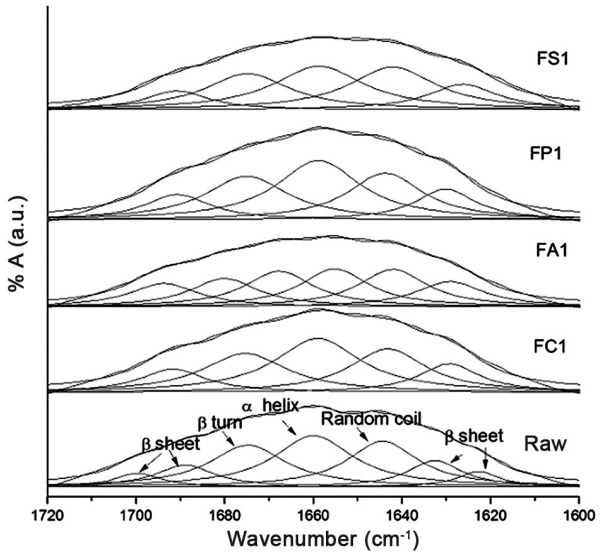

Fig. 4 — Fourier self deconvolution deduced by curve fitting analysis in the region 1720-1600 cm^{-1}

Table 2 — Intensity ratios selected bands of spirulina and its commercial products

Intensity	Significance	Raw	FS1	FC1	FP1	FA1
I_{1545}/I_{3291}	Protein	0.89±0.03	0.83 ±0.03	0.88 ±0.02	0.84 ±0.02	0.57±0.02
I_{2960}/I_{2873}	Protein	1.29 ±0.08	1.24 ±0.08	1.25 ±0.06	1.26 ±0.08	1.15 ±0.07
I_{1545}/I_{1657}	Protein	0.73 ±0.02	0.74 ±0.02	0.73 ±0.02	0.72 ±0.03	0.69 ±0.02
I_{2924}/I_{2853}	Lipid	1.40 ±0.6	1.36 ±0.05	1.38 ±0.05	1.32 ±0.07	1.46 ±0.07
$I_{2960}/I_{2927}+I_{2852}$	Fatty acids	0.55 ±0.01	0.53 ±0.02	0.53 ±0.01	0.51 ±0.02	0.47 ±0.02

The values are the mean ± SE for each group (n=3). The degree of significance was $p < 0.05$

On account of sample FA1 % band area of α helix, β turns and random coil declines and increment in β sheet was watched which is steady with the mechanism of β sheet formation. On account of FP1and FA1 sample there is a noteworthy increment in β sheet, α helix and β turn and abatement in random coil showing protein denaturation because of oxidative change during food processing. This variation in the secondary structure of protein in the samples was responsible for the segregation of the samples which were revealed in PCA scores plots. Comparable outcomes from change in protein secondary structure of spirulina were accounted for by Liu *et al.*[21-22]. In the present study the % area of α helix, β turns and β sheet increments for all commercial samples when contrasted with raw samples except on the FA1 sample α helix, β turn and random coil diminishes.

Figure 5 establishes the consequences of the curve fitting got in the region of fatty acids 3050-2800 cm^{-1}. Table 4 demonstrates the results of the band area of the fatty acids. As observed from the spectra a decline in lipid bands was seen in the sample FP1 and FA1. This might be due to utilization of pretreatment of the commercial sample during sample processing or due to the addition of preservative which brings down the lipid substance. Further the second derivative curve fitting was additionally performed in the distinct region 1200-900 cm^{-1} to determine any variation in polysaccharides of the samples (Fig. 6). Table 5 demonstrates the results of glycogen ~1010 cm^{-1}, glucose ~1035 cm^{-1} and lactic acid ~1127 cm^{-1}. It is observed that % band area diminishes altogether from all samples. This diminishing in glucose is because of oxidation of starch during food processing (such as heating/mechanical pressure) of spirulina. The glycogen and lactic acid increment significantly among the four samples with the exception of FA1 and FP1 which empowers us to separate from different samples which were upheld by PCA scores plot.

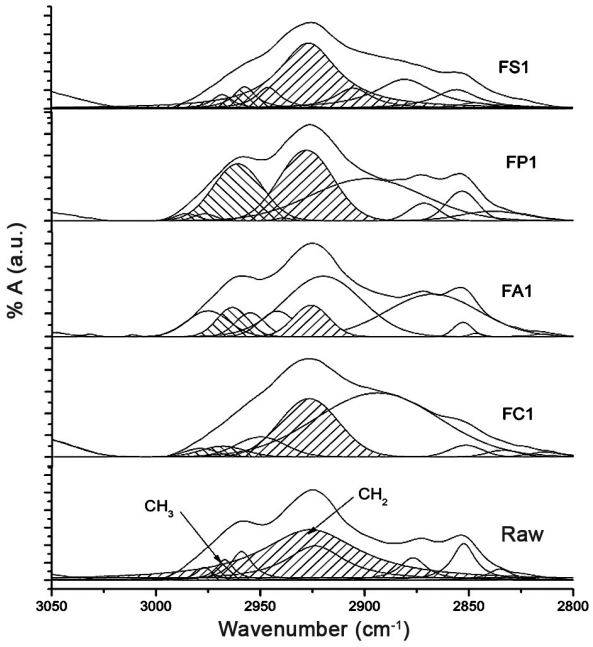

Fig. 5 — Fourier self deconvolution deduced by curve fitting analysis in the region 3050-2800 cm^{-1}.

Table 3 — Results of curve fitting analysis expressed as a function of percentage areas of protein secondary structures and their band assignments for raw and commercial sample of spirulina in amide I region

	Raw		FS1		FC1		FP1		FA1	
Assignments	Wave-number	% Area	Wave-number	% Area	Wave-number	% Area	Wave-number	% Area	Wave-number	% Area
β sheet	1622	4.33±0.08	1626	12.98±0.32	1629	11.94±0.52	1603	11.83±0.22	1629	13.44±0.08
β sheet	1632	9.73±0.21	-	-	-	-	-	-		
Random coil	1644	22.41±0.43	1642	27.20±0.63	1643	22.74±0.37	1643	22.39±0.37	1642	19.89±0.14
α helix	1659	28.20±0.32	1658	28.25±069	1659	32.06±0.62	1659	31.89±0.43	1655	20.20±0.17
β turn	1674	22.29±0.82	1674	22.3±041	1675	22.76±0.48	1675	23.23±0.38	1667	18.98±0.13
β sheet	1689	8.79±0.16	1691	8.60±0.07	1691	7.11±0.09	1690	12.67±0.14	1693	15.03±0.08
β sheet	1699	4.24±0.07	-	-	-					

The values are the mean ± SE for each group (n=3). The degree of significance was $p < 0.05$

Table 4 — Results of curve fitting analysis of fatty acids expressed as a function of percentage areas and their band assignments for raw and commercial sample of spirulina in 3050-2800cm^{-1} region

	Raw		FS1		FC1		FP1		FA1	
Assignments	Wave-number	% Area	Wave-number	% Area	Wave-number	% Area	Wave-number	% Area	Wave-number	% Area
CH2	2928	20.49±0.43	2926	44.32±0.29	2926	23.19±0.42	2927	27.3±0.38	2925	8.17±0.24
CH3	2966	3.63±0.02	2968	3.28±0.02	2968	4.22±0.22	2968	3.22±0.09	2963	6.55±0.31
CH	3032	0.21±0.01	3010	0.00012±0.00001	3023	0.16±0.04	3019	0.01±0.005	3011	0.02±0.01

The values are the mean ± SE for each group (n=3). The degree of significance was $p < 0.05$

Table 5 — Results of curve fitting analysis of carbohydrates expressed as a function of percentage areas and their band assignments for raw and commercial sample of Spirulina in 1200-900 cm^{-1} region

	Raw		FS1		FC1		FP1		FA1	
Assignments	Wave-number	% Area	Wave-number	% Area	Wave-number	% Area	Wave-number	% Area	Wave-number	% Area
Glycogen	1011	5.71±0.07	1010	10.51±0.25	1013	7.51±0.21	1015	7.02±0.17	1018	9.57±0.42
Glucose	1033	28.32±0.14	1024	10.64±0.38	1034	10.31±0.42	1030	1.68±0.06	1031	9.57±0.61
Lactic acid	1127	3.55±0.02	1126	5.22±0.09	1128	3.16±0.04	1128	11.73±0.35	1124	10.53±0.51

The values are the mean ± SE for each group (n=3). The degree of significance was $p < 0.05$

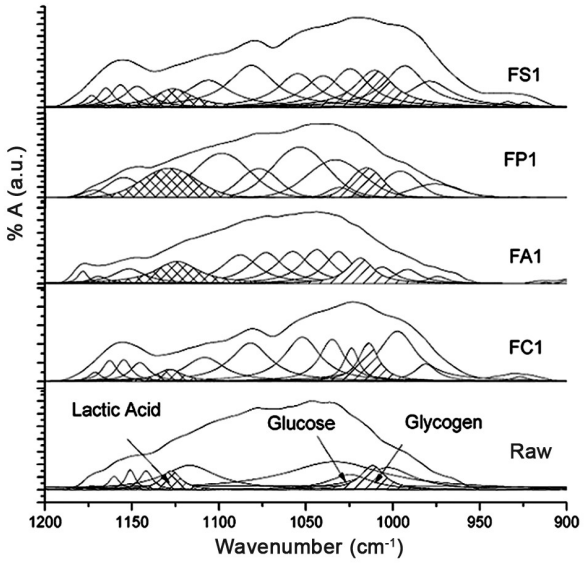

Fig. 6 — Fourier self deconvolution deduced by curve fitting analysis in the region 1200-900 cm^{-1}

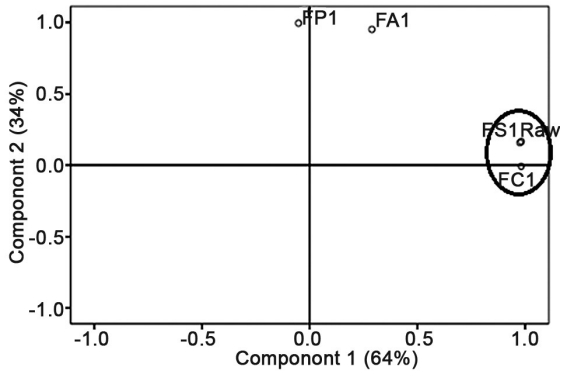

Fig. 7 — PCA scores plot spirulina and its commercial products showing distinct variation obtained by factor reduction method using principle component analysis

3.3 Principle component analysis biochemical compositions

Figure 7 shows the number of component with corresponding eigen values after factor reduction. It shows that only two components have the highest value of eigen value obtained. This two components account for most of the variations in factors obtained after factor reduction. Figure 7 reveals the variation on loading plots against the first two components. This

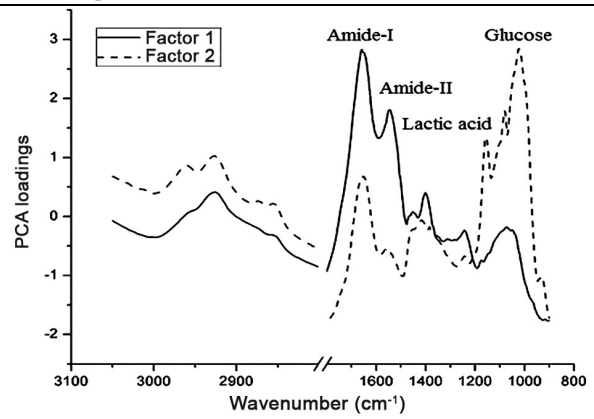

Fig. 8 — Graph showing variation of factor loading obtained from PCA method with the corresponding wave number

2 component is plotted with loading value corresponding to each sample. The inspection of this figure shows that FS1 and FA1 are clustered near raw sample showing no distinct variation on the samples. The observation of the PCA plot shows discrimination of the sample FP1 and FA1. In Fig. 8 the inputs of PCA loadings with the corresponding wave number are plotted. The prominent band of the graph appeared to be highly influenced by the strong loading at 1659 cm^{-1} which corresponds to amide I from factor 1 and 1026 cm^{-1} which corresponds to glucose from factor 2 loading. Other, less intense loading included are, the contribution to amide II and III band. By considering this feature of the factor 1 loading the major influences that separate the samples amide I (64%) from the others. For factor 2 loading, the glucose influences major separation between the samples (34%). Amide III contributes to less extent. This suggests difference in proteins, carbohydrate and lipids which contributes more or less to the clustering. The protein and carbohydrate moiety is the key components that influence the separation of the samples.

4 Conclusions

Noteworthy changes in the biochemical constituents, for example, proteins, lipids and carbohydrates in the spirulina and its commercial products were observed utilizing FTIR spectra. An abatement in protein and increment in lipid, glycogen

is seen from the intensity ratio. The progressions are more affirmed in commercial sample FA1 and FP1 recommending an adjustment in the protein and lipid which happens during food processing. In addition to a decrease at random coils and increase of α helix, β sheet, β turns were observed for the sample FP1 and FA1. Additionally commercial spirulina based food products have shown a significant role in lipid abnormalities indicating the antioxidant property. This plays an effective role in the biochemical changes which are balanced by utilization of glucose leading to decrease from its content. Further the use of the second derivative and factor loading leads to highly accurate prediction and monitoring of food quality related products under stringent industrial standards. Subsequently the utilization of a PCA to the FTIR spectra demonstrated that a distinction was conceivable, the major factors (76%) were clarified by PC1 that isolates amide I and for PC2 (36%) of glucose that isolates from alternate samples. These outcomes demonstrate that the FTIR spectroscopy can be utilized as an extremely dependable instrument for sustenance validation of the carbohydrates based additives. Further application of chemometrics in segregations will put a quantitative premise of justification for the conclusion of the analyst.

Acknowledgement

The author communicates earnest on account of SAIF, IIT Madras for aiding in recording the FTIR spectra.

References

1 Dillonm J C, Phuc A P & Dubacq J P, *World Rev Nutr Diet*, 77 (1995) 32.
2 Pelizer L H, Danesi E D G, Rangel C D, Sassano C E N, Carvalho J C M & Sato S, *J Food Eng*, 56 (2003) 371.
3 Reid L M, O'Donnell C P & Downey G, *Trends Food Sci Tech*, 17 (2006) 344.
4 Cordella C, Moussa I, Martel A C, Sbirazzuoli N & Lizzani C L, *J Agric Food Chem*, 50 (2002) 1751.
5 Dean A P, Sigee D C, Estrada B & Pittman J K, *Bioresour Technol*, 101 (2010) 4499.
6 Murdock J N & Wetzel D L, *Appl Spectro Rev*, 44 (2009) 335.
7 Hirschmugl C J, Bayarri Z E, Bunta M & Holt J B, *Infrared Phys Technol*, 49 (2006) 57.
8 Cakmak G, Zorlu F, Severcan M & Severcan F, *Anal Chem*, 83 (2011) 2438.
9 Toyran N, Lasch P, Naumann D, Turan B & Severcan F, *Biochem J*, 397 (2006) 427.
10 Gupta A D & Karthikeyan S, *Ecotoxicol Environ Saf*, 130 (2016) 289.
11 Ozek N S, Sara Y, Onur R & Severcan F, *Bio Sci Rep*, 30 (2010) 41.
12 Bozkurt O, Severcan M & Severcan F, *Analyst*, 135 (2010) 3110.
13 Karthikeyan S, *Rom J Biophys*, 24 (2014) 109.
14 Bogomolny E, Argpv S, Mordecjao S & Huleihel M, *Biochim Biophy Acta*, 1780 (2008) 1038.
15 Ravi M, Lata De S, Azharuddin S & Paul S F D, *Nutr Diet Suppl*, 2 (2010) 73.
16 Wsowicz E, Gramza A, Heoe Ma, Jelen H H, Korczak J, Maecka M, Mildner-Szkudlarz S, Rudzinska M, Samotyja U & Zawirska-Wojtasiak R, *Pol J Food Nutr* Sci, 13 (2004) 87.
17 Melin A M, Perromat A & Deleris G, *Can J Physiol Pharmacol*, 79 (2001) 158.
18 Ozaki Y, Kaneuchi F, Iwamoto T, Yoshiura T & Iriyama K, *Appl Spect*, 43 (1989) 138.
19 Benedetti E, Teodori Laura, Trinca M L, Vergamini P, Salvati F, Nauro F & Spremolla G, *Appl Spect*, 44 (1990) 1276.
20 Karthikeyan S & Mani P, *Biophysics*, 59 (2014) 321.
21 Liu H J, Xu C H, Zhou Q, Wang F, Li W M, Ha Y M & Sun S Q, *Radiat Phy Chem*, 85 (2013) 210.
22 Jing L H, Qinz S, An L & Ming H Y, *Sci Agric Sinica*, 45 (2012) 4738.