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Characterization of microalgal species through TGA/FTIR analysis: Application to *nannochloropsis* sp.

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ABSTRACT

In this work, the on-line combination of Thermogravimetric Analysis (TGA) and Fourier Transform Infrared Spectrometry (FTIR) has been applied to study the evolution with time of the volatile products evolved in the thermal pyrolysis of *nannochloropsis* sp. in order to characterize the different decomposition steps of this microalgal. The microalgal cells were treated in order to separate the lipid fraction, by breaking the cells and extracting the fraction soluble in hexane, and both fractions, i.e., the extract and the extraction residue, have been also analyzed by TGA/FTIR. The results obtained for the IR spectra of the gases evolved in the pyrolysis of the samples (microalgae, extract and extraction residue) confirm the extraction of compounds present in the microalgae whose decomposition are the responsible of the intensity of this band being related to the lipids present in the microalgae. On the other hand, the results show a different behaviour related to the main absorption bands analysed. The peak related to the C–H absorption band is shifted to higher temperature compared with the peak obtained for the other different functional groups analysed (C═O, CO_2 and C—O—C), showing that the lipid compounds were probably released in the last decomposition stages of the main decomposition step observed in the thermogravimetric analysis.

1. Introduction

In recent years, the study of the different aspects related to the behaviour of microalgae has received renewed interest due to the wide field of application of these microorganisms. Algae cultures have been principally developed as an important source of many products, such as aquaculture feeds, human food supplements, and pharmaceuticals [1–4], and they have also been suggested as a very good candidate for fuel production [5]. The pyrolysis or the combustion of the biomass coming from microalgal cultures can be used as an alternative source of energy [6,7], but also the existence of some species rich in lipids can be exploited as an interesting alternative for biodiesel production [8,9]. Moreover, the installation of bioreactors for microalgal production nearest industrial installations which are the focus of CO_2 emissions can help to reduce such emissions by using CO_2 as a nutrient for the culture [10,11].

Nannochloropsis sp. is a small green alga that is extensively used in the aquaculture industry for growing small zooplankton such as rotifers, but its use has also been recognized for human diet due to its nutritional value, related to its biochemical composition as an excellent source of proteins, carbohydrates, lipids and vitamins. This microalga is well known as a source of different valuable pigments, such as chlorophyll a, zeaxanthin, canthaxanthin and astazanthin [4], produced at high levels. On the other hand, glucose is the dominant sugar in the polysaccharide composition [12]. In the protein, the amino acids aspartate, glutamate and proilina predominate. But this microalga is also recognized as a good potential source of fatty acids such as eicosapentaenoic acid, an important polyunsaturated fatty acid for human consumption for the prevention of several diseases [13].

The ability of the application of Thermogravimetric Analysis (TGA) for the study of microalgal species has been proved by several authors. As an example, Pane et al. [14] applied TGA in an air atmosphere to study the effects of temperature on a marine planktonic alga, reporting the existence of marked differences between the different phases of growth, related to the presence of different molecules produced during the algal growth and to the differences in the thermal properties of these intracellular molecules. These authors differentiate three steps in the weight loss TGA curves. The first stage of weight loss occurs in the 40–180 °C range and corresponds to the loss of free water and water loosely bound to biomolecules. In this process, the cell structure is progressively destroyed, and phenomena such as alteration of lipid structures

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and proteic thermal unfolding occur. The second step occurs in the 180–400 °C range, and involves the decomposition of proteins and carbohydrates. This step produces the main loss of weight. Finally, the third step occurs in the 400–760 °C range and corresponds to the complete oxidation of the organic matter. Peng et al. [15] pyrolyzed two kinds of autotrophic microalgae in TGA equipment in order to investigate their pyrolytic characteristics. These authors also identify three stages of decomposition, i.e., dehydration, devolatilization and solid decomposition, and performed a kinetic study in order to characterize each species. TGA was also used by Zhang et al. [16] for the study of a composite of polyethylene–chlorella.

The on-line combination of TGA and Fourier Transform Infrared Spectrometry (FTIR) has been successfully applied for the study of the evolution with time of the volatile products evolved in the thermal and catalytic pyrolysis of polymers [17,18], showing the ability of this technique to characterize the nature of the chemical compounds which are decomposed in the different degradation steps involved in the pyrolysis of ethylene–vinyl acetate copolymers.

In this work, TGA/FTIR has been applied for the study of the evolution with time of the volatile products evolved in the thermal pyrolysis of *nannochloropsis* sp. for the characterization of the different decomposition steps of this microalgal specie. In this way, the microalgal cells have been treated in order to separate the lipid fraction, by breaking the cells and extracting the fraction soluble in hexane, and both fractions, i.e., the extract and the solid residue, have been also analyzed by TGA/FTIR.

2. Materials and experimental procedure

2.1. Culture condition

The culture was grown in sterilized seawater enriched with f/2 medium nutrients for marine algae [19]. The algae were alga grown photoautotrophically in batch cultures where inorganic CO_2 from the atmosphere was the only source of carbon. Aeration was provided by bubbling air at regular pressure. The temperature was kept at 20 °C.

2.2. Harvesting, lipid extraction and analysis

Cells were harvested by centrifugation, washed with distilled water, and then dried in an oven at 60 °C for 1 day. To obtain information about the ashes content of the microalgae a calcination at 800 °C during 2 h have been performed, yielding an ashes content of 7%. On the other hand, lipid components (extract) were obtained by pulverization of cell powder in a mortar and then extraction with *n*-hexane. The extract was dried under vacuum to remove the solvent and was then saponified and methylated according to the procedure of Zhu et al. [20]. The fatty methyl esters were determined by using the GC–MS technique.

Samples of microalgae, as well as of extract and residue, of around 3 mg were pyrolyzed in an N_2 atmosphere (99.999% minimum purity) using a TGA Netzsch TG209 at a heating rate of 35 °C/min. The heating rate was selected high enough to ensure the quality of the FTIR data obtained [21]. To ensure the measurement of the actual sample temperature, a calibration of the temperature was performed using the Curie-point transition of standard metals, and a parabolic correction was applied. The output of the inert gas from the TGA was connected to a Bruker Tensor 27 FTIR spectrometer through a heated line, as described in the bibliography [22]. The balance adapter, the transfer line and the FTIR gas cell can be heated until 523 K, thus avoiding the condensation of the less volatile compounds. On the other hand, the low volumes in the thermobalance microfurnace, transfer line and gas measurement cell permit low carrier gas flowrates to be used and allow for good detection of the gases evolved in the pyrolysis process. In all the experiments, the transfer line and the gas measurement cell were maintained at 473 K.

3. Results and discussion

3.1. TGA results

Fig. 1 shows the weight loss curves obtained by TGA curves and the derivative of the TGA curves (DTG) obtained for the *nannochloropsis* sp. microalgae and for the non-soluble residue and the extract, respectively, obtained by application of the breaking and extraction of the cells in the experimental conditions described in the previous section. As can be seen, in good agreement with the bibliography [14–16], the TGA and the DTG corresponding to the microalgae show three main decomposition steps (i.e., <180 °C corresponding to dehydration, 180–540 °C corresponding to devolatilization and >540 °C corresponding to the slow decomposition of the solid residue resulting from the previous step), the results obtained in this work permit us to observe that the main decomposition step, which occurs in the 180–540 °C range, is actu-

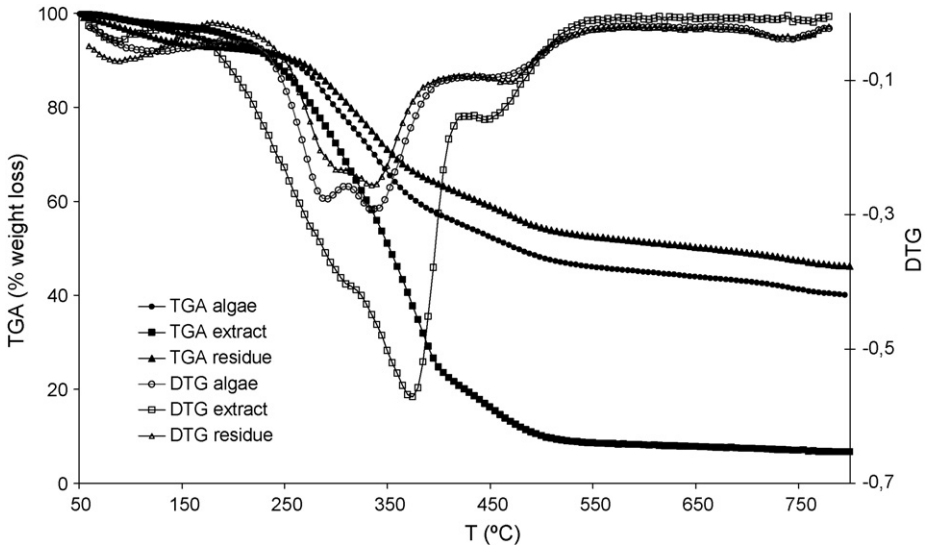

Fig. 1. Thermogravimetric curves for the decomposition of algae, extract and residue samples.

ally a very complex process, involving at least three overlapped steps, such as the shoulder and overlapped peaks observed in the corresponding DTG curve. According to Fig. 1, the temperatures of maximum decomposition rate (i.e., the DTG-peak temperatures) observed for the microalgae pyrolysis are:

- First step (dehydration): 125 °C.
- Second step (devolatilization): three overlapped peaks at 290, 340 and 460 °C.
- Third step (solid residue decomposition): 740 °C.

In the following paragraphs, it is important to clearly distinguish between the two terms "solid residue", which refers to the non-volatile material remaining in the TGA pan as the temperature increases, and the "extraction residue", which refers to the non-soluble fraction obtained after extracting the microalgal specie with hexane as a solvent. The extract is the "crude extract" and contains the lipidic fraction of the microalgae as well as other components, which are soluble in hexane. As can be seen in Fig. 1, the comparison among the TGA curves of microalgae, extraction residue and extract shows a clear difference in the amount of solid residue obtained after the second decomposition step. At 600 °C the extraction residue yields a solid residue of around 51%, the microalgae sample yields 45% of solid residue, and the extract yields around 8% of solid residue. These results are in good agreement with the value of around 14.6% obtained for the percentage of extract obtained after the extraction with hexane: the amount of extract fraction can be estimated as follow:

$$\%\ \text{solid residue from the microalgae} = (\%\ \text{extract}) \cdot \left(\frac{\%\ \text{solid residue from extract}}{100}\right) + (\%\ \text{residue}) \cdot \left(\frac{\%\ \text{solid residue from residue}}{100}\right)$$

$$100 = \%\ \text{extract} + \%\ \text{residue}$$

And considering the values of solid residue obtained at 600 °C, the amount of extract calculated is 14%; very close the experimental value of 14.6%.

As can be seen in Fig. 1, the DTG curve corresponding to the extract does not exhibit the peak at $T > 540$ °C, whereas the residue shows a DTG peak at the same temperature as the microalgae. Therefore, in the TGA/FTIR study, the IR bands corresponding to the gas evolved at this decomposition step correspond to the non-soluble compounds in hexane, probably those of the cellular walls and others. As it will be shown in the following paragraphs, according with the TGA/FTIR results, this step could be related to the decomposition of mineral matter.

The "crude extract" containing triglycerids and other components soluble in hexane, has been saponified, methylated and analyzed by GC/MS, following the procedure described by Zhu et al. [20], and the corresponding fatty acid methyl esters have been quantified. In this way, the analysis of the triglyceride content of the extract indicates the existence of 38.4% of fatty acid methyl esters, representing 5.6% of the original microalgae. These triglycerides are transferred from the microalgae to the extract fraction during the extraction process. In accordance with Fig. 1, the pyrolysis of the extract shows the existence of the main weight loss (87.2%) in the range of 140–500 °C, corresponding to three overlapped processes which occur in the following ranges: 140–240 °C, with around 41.5% weight loss, 240–400 °C, accounting for around 21.1% of weight loss, and 400–500 °C, with around 14.6% of weight loss. Therefore, as it can expected, beside triglycerides, other hexane-soluble compounds appear in the extract. However, the comparison between the observed weight loss in the TGA curves and the results of the analysis of triglycerides in the extract do not permits to clearly assign the different overlapped peaks to the decomposition of specific compounds.

The main decomposition step of microalgae and extraction residue shows very similar shapes in the respective TGA and DTG curves, and the main difference is the relative importance of the different overlapped processes. However, the comparison of these curves with those corresponding to the extract indicates that, not only the relative importance of the overlapped peaks is different, but also some slight displacement of the DTG-peak temperatures can be observed.

With respect to the first weight loss observed in the curves of Fig. 1, the differences among the three samples are the peak temperature and the wideness of the interval of weight loss. Both aspects are related to the location and the strength of the intermolecular bonds between water molecules and the solid structure, which determine the ease of water elimination.

3.2. TGA/FTIR results

Fig. 2 shows, as an example, the three-dimensional (3D) diagrams for the microalgae decomposition, which show the absorbance corresponding to the vibrational modes of the different chemical bonds and functional groups corresponding to the gases evolved in the TGA furnace at each temperature versus the wavenumber and versus the time. Crossing the 3D diagrams in a parallel way to the wavenumber axis, the different maxima of absorbance obtained at each time (or temperature) for the different vibrational modes (i.e., at each wavenumber) can be obtained, whereas crossing the diagrams in a way parallel to the time axis permits to the IR spectra corresponding to the gases evolved at each time (or temperature) temperature to be obtained, thus indicating the type of the chemical bonds or functional groups present in these gases.

The 3D diagrams obtained for the microalgae and the extraction residue are quite similar, a fact, which is in agreement with the low percentage of extract in relation to the original microalgae (14.6%). The more important difference observed in the 3D diagrams corresponding to the different samples (i.e., microalgae, residue and extract) is the higher intensity in the region of 3153–2775 cm^{-1} in the extract in comparison with the intensity of this region in the microalgae and extraction residue spectra. Another important characteristic in the spectra of the three samples is the presence of intense bands in the region 2400–2300 cm^{-1}. These bands are more

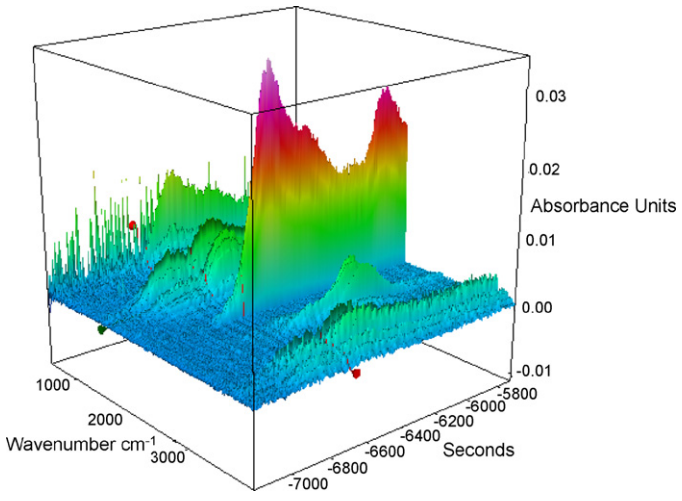

Fig. 2. 3D IR spectrum of microalgae.

intense in the cases of microalgae and extraction residue. Some differences also appear in the 1900–1400 cm^{-1} region, which begins afterwards in the extract case, also showing less intense bands.

On the other hand, the 3D diagrams reflect the existence of groups of absorption bands with very similar trends (same number of maxima at the same temperatures), thus indicating when the maximum decomposition rate of some sample components occurs, yielding the corresponding IR spectra of the gases evolved. The interpretation of such spectra is quite difficult for main reasons: firstly, because the sample which is being decomposed is a complex substance, which originates a complex mixture of volatile compounds, but also because the overlapping of processes which causes that the IR spectra corresponding to each absorbance maximum reflects the adsorption bands of the compounds obtained in such maximum, but also those corresponding to the compounds associated to the tails of the overlapped peaks, i.e., corresponding to different decomposition processes.

According to Fig. 1, five maxima of the generation of volatile products can be found in the corresponding 3D diagram of Fig. 2. These maxima are coincident with the maxima of absorbance observed in the scale of relative time of −6870.48 s (first decomposition step, at around 132 °C), −6605.26, −6547.32 and −6323.82 s (three overlapped processes of the second – main – decomposition step, at around 292, 327 and 462 °C, respectively) and −5860.26 s (third decomposition step, at around 743 °C). Fig. 3a shows the IR spectra corresponding to the gases obtained at these times in the microalgae pyrolysis. The comparison among these spectra reveals the existence of many common bands, with the main differences being in the 3250–2650 cm^{-1} range. The IR spectrum corresponding to the first decomposition step (−6870 s, Fig. 3a) is practically coincident with the IR spectrum of water obtained with the same equipment, and shows the bands at around 1500 cm^{-1} corresponding to bending and in the 4000–3500 cm^{-1} range corresponding to those in plane stretching of free –OH groups. This result was expected, and is in good agreement with the bibliography previously mentioned [15,16]. This spectrum also shows an incipient band at 2341 cm^{-1} corresponding to the presence of some CO_2.

Fig. 3b shows the IR spectra corresponding to the gases obtained in the three overlapped peaks which occur during the main decomposition step (−6605.26, −6547.32 and −6323.82 s, i.e., 292, 327 and 462 °C). The different IR spectra shown in Fig. 3b are very complex, and show a lot of absorption bands. For example, the adsorption bands of water can be observed, thus indicating that water is being still generated, but it seems that more vibrational modes contribute to the bands in the range of 1800–1200 cm^{-1}. Moreover, a noticeable increase of the peak at 2341, corresponding of CO_2 can be observed, as well as the appearance of absorption bands in the regions of 700, 1250–850 and 3100–2650 cm^{-1}. Fig. 3b has been used to show the possible functional group assignation more graphically, similarly to the IR band assignation reported by Yan et al. [23].

An analysis of the different IR spectra obtained in Fig. 3b for the main decomposition stage, reveals the existence of the absorption bands characteristic of these different bonds:

- C=O: The main characteristic of the IR spectra of carbonylic compounds (aldehydes, acids, etc.) is the strong C=O stretching absorption band in the region of 1870–1540 cm^{-1}. In the case of esters, this band appears in the 1750–1735 cm^{-1}.
- C—O—C: corresponding to ethers. These stretching vibrations produce a strong band in the 1200–900 cm^{-1} region [23–25].
- C—H: absorption bands characteristic of the vibrations of C—H bonds, as an example, 2960 and 2875 cm^{-1} correspond to the asymmetric and symmetric vibrational modes of methyl groups, respectively, and 2929 and 2850 cm^{-1} correspond to the asymmetric and symmetric vibrational modes of methylene groups, respectively.

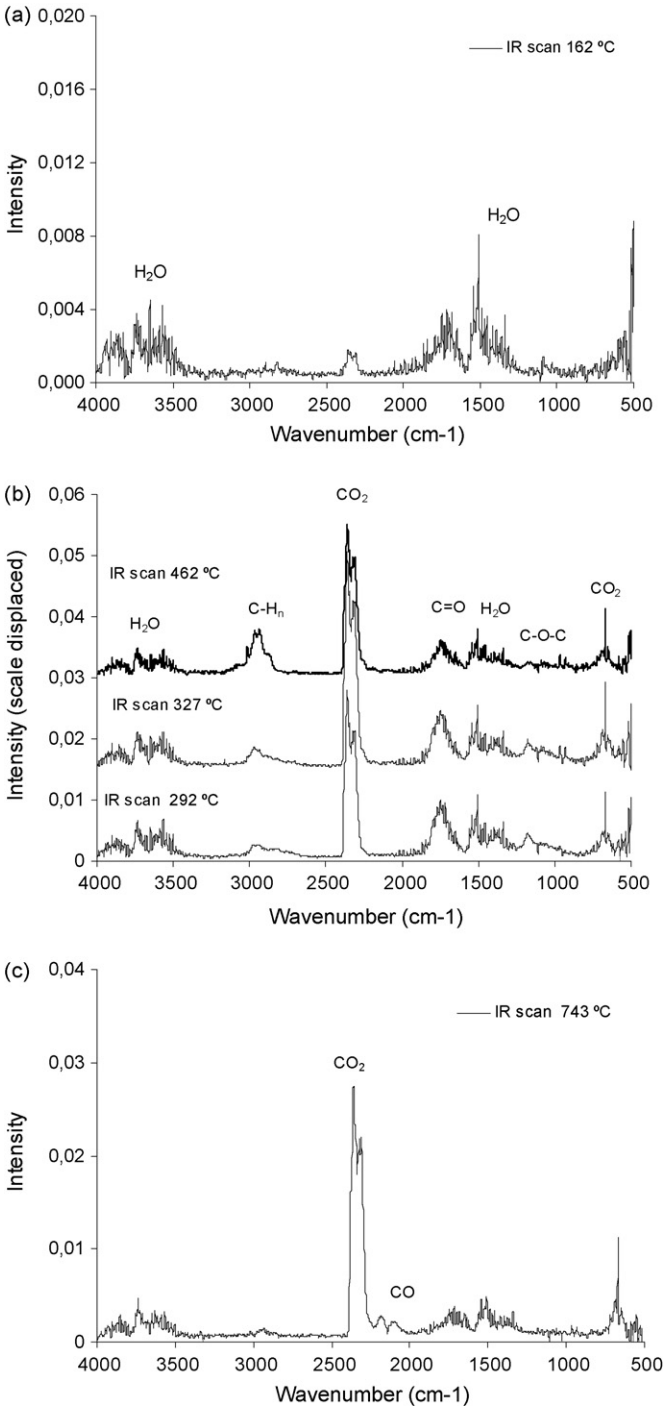

Fig. 3. IR spectrum of the products from pyrolyzing the microalga for the (a) dehydration step (b) main degradation step and (c) solid residue decomposition.

Finally, Fig. 3c shows the IR spectra corresponding to the last decomposition stage observed in the microalgae pyrolysis (Fig. 1). This spectra shows the formation of water and higher amounts of CO_2 (2357 and 2309 cm^{-1}) and CO (2172 and 2097 cm^{-1}). This step could be related to the decomposition of mineral matter, as carbonates.

The results obtained for the IR analysis of the gases evolved in the pyrolysis of the extract can be observed in Fig. 4. Similarly to

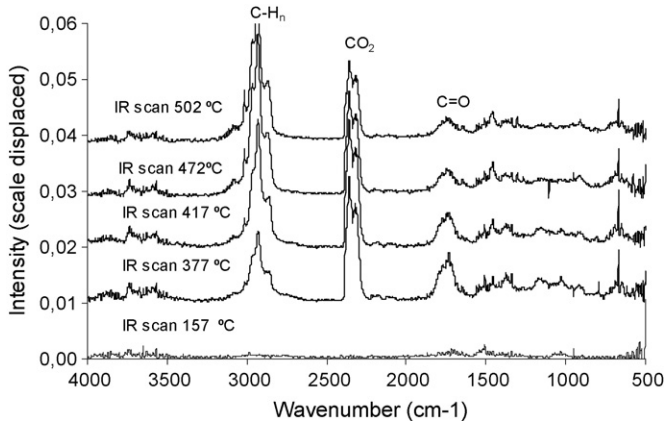

Fig. 4. IR spectrum of the products from pyrolysis the extract for the dehydration step (IR scan 157 °C) and main degradation step (IR scan at 377, 417, 472 and 502 °C).

the IR data treatment for the microalgae, the IR spectra for the main decomposition stages is shown, i.e., the first step (dehydration process, at around 158 °C) and the main decomposition step (at around 378, 418, 473 and 503 °C, respectively). Moreover, one additional IR spectra has been included because of the existence of different overlapped processes in the main decomposition step. The IR spectra results corresponding to the first stage decomposition of extract show the same behaviour observed in the first decomposition stage of the microalgae. For the IR spectra corresponding to the main decomposition step observed in the TG, the presence of the IR band characteristical of the different functional groups previously commented for the microalgae can be seen. However, in the extract, important differences related to the intensity of these different groups can be observed in comparison with the results obtained in the microalgae analysis. In the IR analysis of the gases evolved during the microalgae pyrolysis, the more intense band for the main decomposition stage is that associated to the CO_2, but in the extract, the IR analysis shows that the intensity of the vibration associated to C—H bonds in methyl and methylene groups is more marked than that obtained with microalgae.

Fig. 5 shows the IR spectra corresponding to the gases obtained in the pyrolysis of the extraction residue. This figure shows the different IR spectra corresponding to the first decomposition step (at around 123 °C), the main decomposition step with three overlapped peaks (at around 318, 433 and 498 °C, respectively) and the last decomposition step (at around 734 °C) observed in the TG analysis. The different IR spectra shown in Fig. 5 are very similar to those obtained for the analysis of the microalgae and the different IR bands corresponding to the bonds previously commented can be observed too. In this case, the same considerations commented for the microalgae can be used to explain the different IR spectra.

We consider that the differences observed in the IR spectra can be related to the presence in the gases evolved from the TGA of the products of decomposition of some microalgal components such as proteins, saccharides and lipids (membrane lipids, storage lipids, etc.). When the temperature increases, the decomposition products of these compounds are present in the gas evolved in different proportions and the IR bands characteristic of these products can be observed. For this reason, in order to identify the possible decomposition sequence of these components and to assign the different IR bands analysed as characteristic of these compounds, we have obtained the TG-FTIR of some substances selected as standards of a saccharide (glucose), a lipid (tripalmitine), and an aminoacid (glutamine). Fig. 6 shows the IR spectra of these reference compounds and the possible assignation of the main absorption bands observed. The TGA of these compounds show an important decomposition step and the spectra have been obtained from the gases evolved at the temperature corresponding to the maximum decomposition rate for each standard (250 °C for glucose, 345 °C for glutamine, and 450 °C for tripalmitine). For the lipid spectra two important regions can be observed: 2850–2970 cm^{-1} corresponding to the C—H stretching and ~1740 cm^{-1} associated with the vibration of C=O of ester functional groups. The IR spectra of glucose shows that the intensity of the region 2850–2970 cm^{-1} presents a lower intensity than the same region for the lipid spectra and an important absorption band appears in the 2250–2400 cm^{-1} region corresponding to CO_2. The intensity of this latter band in the lipid spectra is insignificant. On the other hand, other important absorption bands in the IR spectra of the glucose appear in the 1580–1850 cm^{-1} region, corresponding to C=O stretching, and in the 1000–1200 cm^{-1}, corresponding to C—O—C bonds. For the IR spectra of Glutamine the more important adsorption band appears in the CO_2 region. These experimental observations suggest that the presence of the absorption bands in the C–H region are probably intimately related with the lipid components and these bands could be used to monitor the sequence of decomposition of these compounds.

In order to obtain the possible decomposition sequence of compounds such as lipids, the different profiles of the IR intensity of the main functional groups of gas products obtained in the

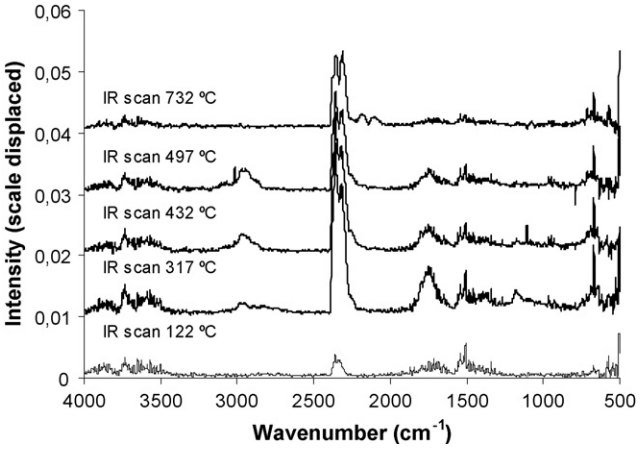

Fig. 5. IR spectrum of the products from pyrolysis, the residue for the dehydration step (IR scan at 122 °C), main degradation step (IR scan at 317, 432 and 497 °C) and solid residue decomposition (IR scan at 732 °C).

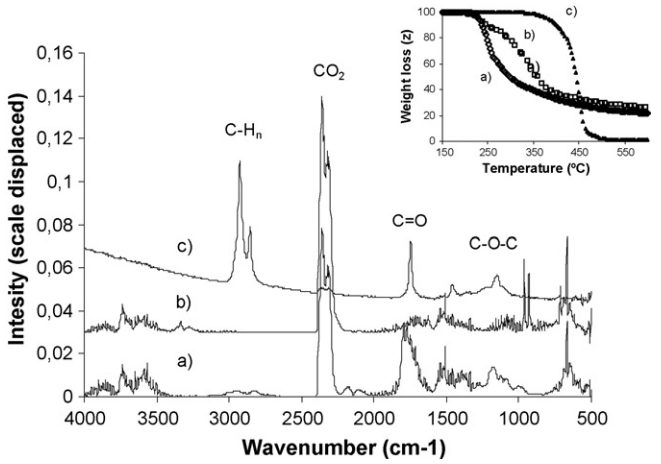

Fig. 6. TG curves and IR spectrum of the products from pyrolyzing of (a) glucose, (b) glutamine and (c) tripalmitine. IR spectra obtained at the temperature of maximum decomposition rate for each compound (250 °C for glucose, 345 °C for glutamine and 450 °C for tripalmitine).

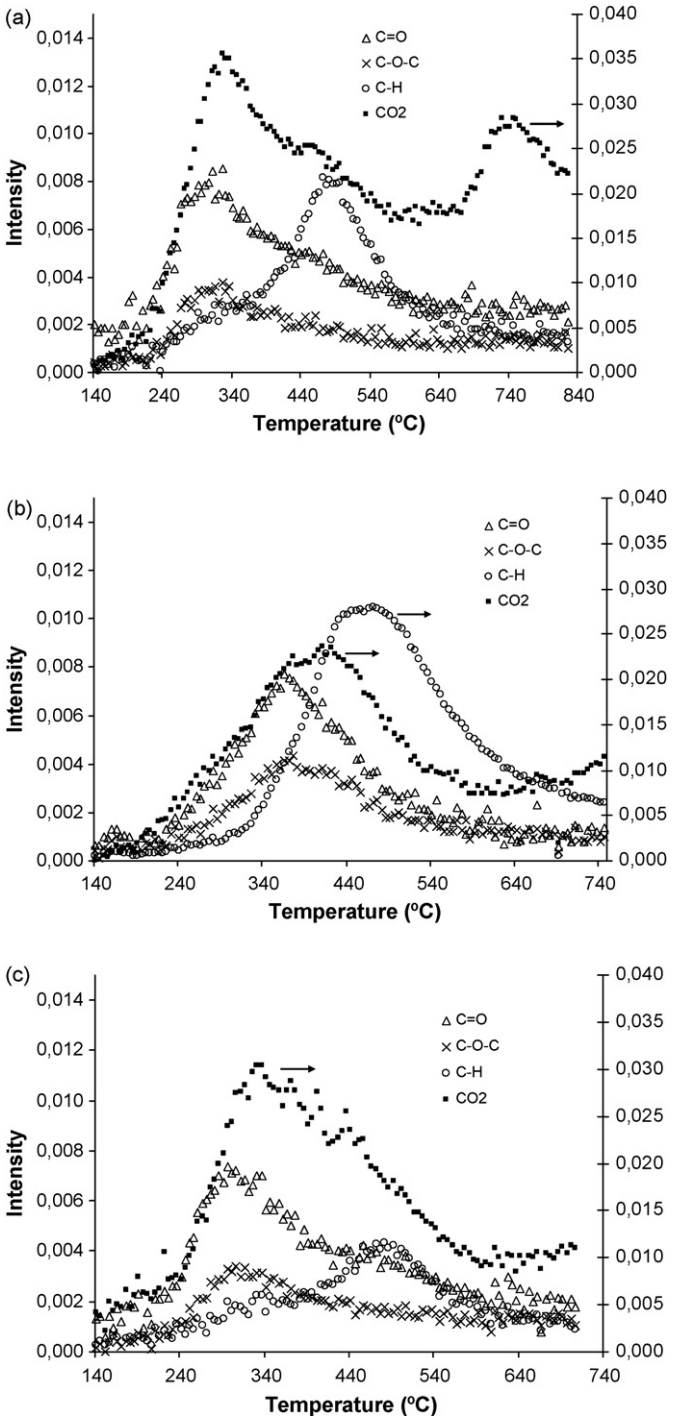

Fig. 7. FTIR profiles of the main IR absorption bands of the gas products obtained in the pyrolysis of (a) microalgae, (b) extract, and (c) residue.

pyrolysis of microalgae, extract and extraction residue are plotted in Fig. 7. In this figure we have plotted the evolution with the temperature of the intensity of the bands associated to CO_2 (2358 cm^{-1}), C—H (2929 cm^{-1} corresponding to the asymmetric vibration of C—H in methylene groups), C=O (1739 cm^{-1}) and C—O—C (1150 cm^{-1}). Important differences in the evolution of these bands can be observed. For the microalgae and the extraction residue, the CO_2 profile shows two peaks where the maximum formation of this compound occurs. These different peaks can be associated to the main decomposition step and the decomposition of the solid residue (last decomposition step) observed in the thermogravimetric analysis of the microalgae and residue. For the extract, only one peak can be observed for the intensity associated for the CO_2 band. This result is in agreement with the behaviour observed for the TG decomposition of the extract, in this case, the last decomposition step corresponding to the solid residue has not been observed. The evolution of the intensity of the bands associated to C=O and C—O—C is very similar for the three samples analyzed (microalgae, extract and residue) and only one peak can be observed. The time corresponding to the maximum of this peak is practically coincident with that observed for the CO_2 evolution and could suggest that the releasing of CO_2 was mainly caused by the cracking and reforming of functional groups as for example carboxyl groups (C=O) [24]. Finally, the evolution for the intensity of C—H, corresponding to the asymmetric vibration of C—H in ethylene groups, shows only one peak for the different samples analysed. In this case, this peak is shifted to higher temperature compared with the peak obtained for the other different products analyzed, showing that these compounds were released in the last decomposition stages of the main decomposition step observed in the thermogravimetric analysis.

Another important factor to analyze is the intensity of these different bands for the three samples analyzed. In the microalgae and extraction residue sample, the intensity of the band associated to the CO_2 is more intense than the other bands analysed for all temperature ranges, however, a different behaviour can be observed in the extract sample. In this sample, while the IR spectra corresponding to the first decomposition peaks at the main degradation step present an important intensity of the band corresponding to CO_2, this band diminishes the intensity for the last decomposition stages of the main degradation step. Contrarily, the intensity corresponding to the vibration of C—H is more important than that associated to the CO_2 for the last stages of the main decomposition step. On the other hand, the comparison between the microalgae and the extraction residue sample shows that the intensity of the band corresponding to C—H is more important in the microalgae than in the residue, this fact together with the increased intensity of the band corresponding to C—H in the extract, confirm the extraction of lipids present in the microalgae whose decomposition is that responsible for the intensity of this band.

4. Conclusions

The study of the evolution with time of the volatile products evolved in the thermal pyrolysis of *nannochloropsis* sp. with the on-line combination of TGA and FTIR has permitted the different decomposition steps of such microalgal species to be characterized. The decomposition of the microalgae occurs in three important degradation steps that can also be observed in the IR spectra. The comparison between the microalgae and extraction residue sample shows that the intensity of the band corresponding to C—H is more important in the microalgae than in the residue. This fact together with the greater intensity of the band corresponding to C—H in the extract, confirm the extraction of compounds present in the microalgae whose decomposition is responsible for the intensity of this band. The analysis of the IR spectra of the gases evolved in the pyrolysis of the samples shows a different behaviour related to the main absorption bands analysed. The peak related to the C—H absorption band is shifted to higher scans (i.e., higher temperature) compared with the peak obtained for the other different products analyzed (C=O, CO_2 and C—O—C), showing that the C—H compounds were released in the last decomposition stages of the main decomposition step observed in the thermogravimetric analysis. A TG-IR analysis of compounds such as glucose, tripalmitine and glutamine suggests that the C—H absorption band could be due to the lipid components of microalgae. According with the results obtained in this

work, the different biochemical components of the microalgae seem to be decomposed in the following order: firstly polysaccharides and proteins and finally lipids. These considerations could be used to estimate the approximate lipid proportion of a given microalga; however, deeper studies must be carried out to clarify this point.

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