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ACYL LIPIDS OF THREE MICROALGAE

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Key Word Index—*Isochrysis galbana*; Prymnesiophyceae; *Phaeodactylum tricornutum*; Bacillariophyceae; *Porphyridium cruentum*; Rhodophyceae; acyl-lipid composition; polyunsaturated fatty acids.

Abstract—Acyl-lipid composition of Isochrysis galbana, *Porphyridium cruentum* and *Phaeodactylum tricornutum* from stationary-phase cultures has been analyzed by TLC and GC. Additionally, *P. tricornutum* from an outdoor tubular photobioreactor was also studied. Neutral (NLs) and glyco (GLs) lipids were found in similar amounts of ca 40–45% each, with phospholipids (PLs) representing ca 10–20%. The major lipid classes were triacylglycerol (TAG), monogalactosylaylglycerols (MGD), and galactosylacylglycerols (DGD), usually in that order. The *P. tricornutum* biomass taken from the bioreactor showed a distinctive acyl-lipid composition. GL content was nearly twice (56%) that of the indoor culture (31%) and NL content (31%) was nearly half that of the indoor culture (54%). These changes mainly involved TAG and MGD. The fatty acid composition of lipids in the outdoor culture generally remained unaffected, except for MGD, DGD, phosphatidylcholine (PC) and phosphatidylethanolamine (PE), in which eicosapentaenoic acid (EPA) content was greatly increased.

TAG, the main class of lipids, always contained a high proportion of EPA, 16:0 and 16:1. In the typical (SQD) composition, 14:0, 16:0 and 16:1 accounted for around 70% of fatty acids, with small amounts of polunsaturated fatty acids (PUFAs). The lipid classes which typically had the highest PUFA content in the microalgae were MGD and DGD. In *P. tricornutum*, these lipids characteristically contained a large proportion of 16:1, 16:2, 16:3 and 16:4. PC, which was composed of the main C_{16} fatty acids and the major PUFAs, was usually the most abundant phospholipid. Phosphatidylgycerol showed an accumulation of 16:1 (probably a mixture of 16:1n7 and 16:1n3*trans*) as a distinctive feature. Phosphatidylinositol was characterized by 16:0 and 16:1 (and 14:0 in *I. galbana*), which together accounted for over 50% of fatty acids, and significant presence of the main PUFAs. There was no consistent fatty acid pattern for PE in the three microalgae studied. PE was exceptional in *I. galbana*, containing 61% docosahexaenoic acid.

INTRODUCTION

Fatty acid composition of microalgae is reasonably well documented. Nevertheless, much less work has been done on the individual classes of lipids found in them [1, 2] or on the fatty composition of these lipids [2]. For example, a review of the bibliography for ten phytoplankton classes [2] reported lipid class analyses for fewer than ten species. Likewise, with exceptions [3–7], most reports are also insufficiently detailed (e.g., Ref. [8]).

On the other hand there is a growing interest in polyunsaturated fatty acids (PUFAs), especially for arachidonic acid (AA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) due to their involvement in human health. Microalgae are current potential sources of these long-chain PUFAs [9, 10] and our group are involved in stages from strain selection [11, 12] to PUFA purification [13, 14], in order to provide a cheap and reliable source to satisfy pharmaceutical requirements. In this regard it is recognized that both the localization of PUFA (e.g., EPA) inside the lipid pool and the companion fatty acids are critical aspects for the purification process.

The objective of the present work was to provide data on the types of lipids, content of each lipid class and fatty acid composition of each type, in three microalgae (*Isochrysis galbana*, *Porphyridium cruentum*

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and *Phaeodactylum tricornutum*) with potential use for production of highly valuable PUFAs (i.e., EPA, AA and DHA). This work provided new information on *I. galbana*, reevaluated controversial information on *P. cruentum*, compared lipid composition between indoor and outdoor cultures of *P. tricornutum*, and presents data, (e.g., on monoacylglycerols, (MAG) and diacylglycerols (DAG)) that confront or modify the current status of microalgal lipid composition.

RESULTS AND DISCUSSION

As a consequence of the methods used (see Experimental), the following data refer exclusively to saponifiable matter (i.e., acyl-lipids); non-saponifiable matter was excluded from quantitation. This is especially relevant for lipid fractions, because, as is well known, they always contain some non-saponifiable lipids (e.g., sterols, pigments and hydrocarbons) [1, 15].

Lipid composition

Some of the lipid classes. DAG, triacylglycerols (TAG) digalactosyldiacylglicerols (DGD) and monogalactosyldiacylglycerols (MGD) resolved into more than a single band after one-dimensional TLC (but not in two-dimensional TLC). For example, TAG in *I. galbana* produced four bands. each with significant differences in fatty acid composition (data not shown). Similar results have been reported before [16]. All such bands in a lipid class were combined to obtain a single fatty acid composition for each.

Analyses of saponifiable lipid fractions showed roughly similar proportions of each fraction in the three indoor cultured species (Fig. 1a–c). The outdoor culture of *P. tricornutum* exhibited a very specific lipid composition (Fig. 1d) that will be discussed later.

Neutral lipids (NLs) and glycolipids (GLs) were always the main fractions, together accounting for ca 80–85% of the saponifiable lipids, with phospholipids (PLs) constituting ca 15–20% (Fig. 1a–c). Therefore, polar lipids normally formed the main portion of acyl-lipids as usually reported [4, 5, 7, 17]. NLs and GLs were found in approximately analogous amounts of ca 40% of the saponifiable lipids (Fig. 1a–c). More variation was observed in the proportions of lipid classes (Fig. 1a–c), although, it might be generalized that TAG, MGD and DGD, were usually, in that order, the main lipid classes in the three microalgae examined. MGD usually followed TAG in importance, except in *P. cruentum*, where DGD was the main GL (Fig. 1b). The individual PL classes were always found in minor amounts of less than 10%, usually ca 4% (Fig. 1a–c).

TAG has usually been reported in a significantly lower proportion than MGD, whilst our data clearly contradict this (Fig. 1a–c). Notwithstanding, a few studies have shown TAG as the main lipid class, for example, in *Chroomonas salina* [18], in an Antarctic Prymnesiophyte [19], in *Pavlova lutheri* [7], in *Nannochloropsis* [6] and in *P. cruentum* under certain culture conditions [4]. TAG often accumulates in times of nutritional excess or under stress. Our cultures were taken from the stationary phase when some nutritional resource was depleted, so microalgae accumulate storage lipids (mainly TAG). When the culture is young, lipid composition changes considerably, as will be discussed later. MAG and DAG were present in all microalgae in significant quantities between 3–19% of acyl-lipids (Fig. 1a–c). The presence of MAG and DAG is controversial because these lipid classes are not usually detected (e.g. [17, 20]). Some workers follow a procedure that discards, *a priori*, the possibility of detection of these lipid classes (e.g. [5, 7]). Nonetheless, we think that, in most cases, the lack of detection of MAG and DAG is likely to be a question of amount. Many authors used ca 10 mg of lipid extract (e.g. [17]), while we have taken between 20–40 mg of lipids producing between 9–18 mg of NLs. Therefore, we were probably able to detect MAG and DAG simply because we have loaded TLC plates with a larger amount of NLs. Moreover, MAG and DAG were previously detected, although not separated. in *P. cruentum* by others [4]. On the other hand, these lipid classes could be the result of lipid degradation by lipases. However, when hot (50–60°) *iso*-propanol was used as extracting solvent to inactivate those enzymes [21], MAG and DAG appeared in similar quantities to those reported here.

MGD is usually the major GL in red and green macroalgae [22], as well as in some microalgae [5, 23]. Nevertheless, a wide range of GL contents has been reported in macroalgae [22–24] and in microalgae [5, 6, 18, 23]. Moreover. in some microalgae, the proportion of DGD was higher than MGD, as in *Chroomonas salina* [18] and *P. cruentum* (Fig. 1b).

Among the PLs, phosphatidylcholine (PC) was the most abundant in *I. galbana* and *P. cruentum* (Fig. 1a and b) and phosphatidylethanolamine (PE) in the indoor culture of *P. tricornutum* (Fig. 1c). Results for PL in *P. cruentum* were roughly in agreement with others previously published [4, 25]. PC was also observed to be the main PL in *I. galbana*, as was the case in one Prymnesiophyte [19], but PC was undetected in another Prymnesiophyte, *Pavlova lutheri* [7]. Moreover, it has been suggested that betaine lipids may substitute for PC in the Prymnesiophytes [26] but we have not detected these lipids in our samples. The main PL reported in the diatom *P. tricornutum* [5], although unidentified lipid might be PE. This would agree with our data for the indoor culture (Fig. 1c), but not for the outdoor culture, where PC was also the major PL (Fig. 1d). In the marine diatom, *Navicula pelliculosa*, PC was also the main PL [16]. Therefore, although PC seems usually to be the main PL in microalgae [6, 16, 18, 19], some variation may be expected due to species-specificity or to culture conditions [4, 27].

A dramatic change was observed in lipid composition between indoor and outdoor cultures of *P.*

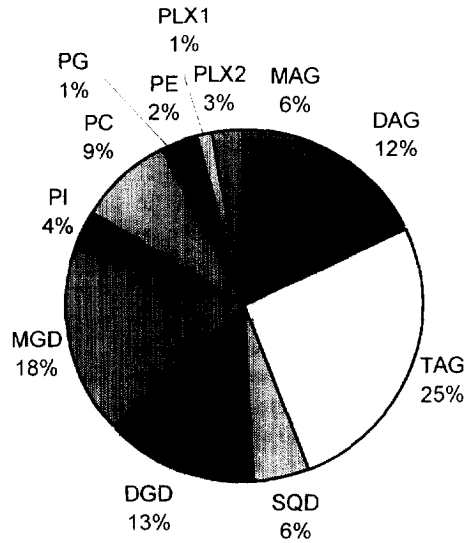

Figure 1a

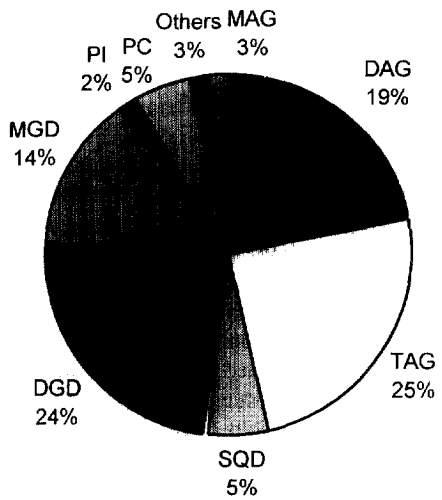

Figure 1b

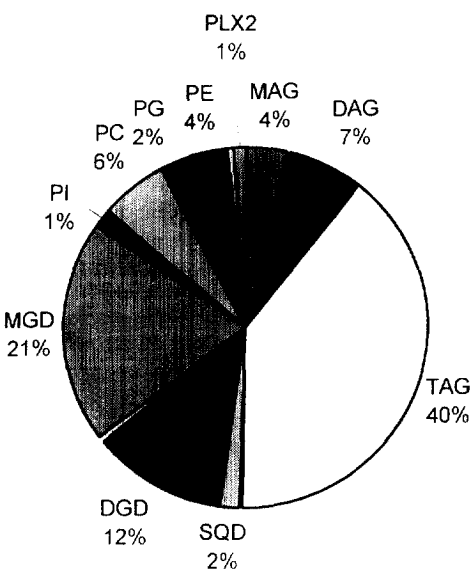

Figure 1c

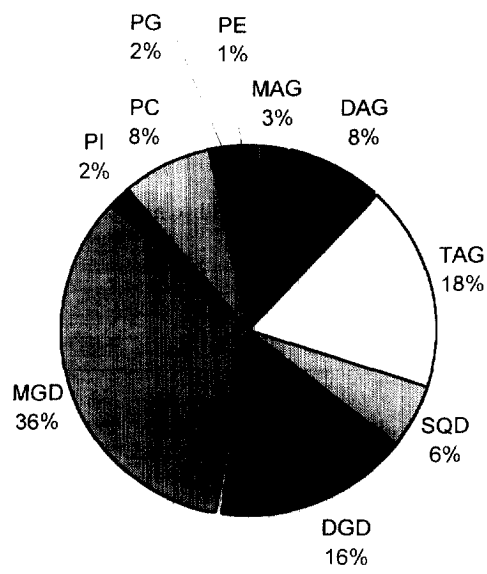

Figure 1d

Fig. 1. Lipid composition (wt% total fatty acids) of three microalgae: (a) *Isochrysis galbana*, (b) *Porphyridium cruentum*, (c) *Phaeodactylum tricornutum* (indoor culture), and (d) *P. tricornutum* (outdoor culture).

tricornutum. The NL fraction shifted from 51 to 29% and the GL fraction from 35 to 58%, in the indoor and outdoor cultures, respectively, although the PL fraction remained virtually unchanged (Fig. 1c and d). TAG went down from 40 to 18%, MGD up from 21 to 36%, in the indoor and outdoor cultures, respectively (cf. Fig. 1c and d) and, although the total amount of PLs did not vary, PE was the main PL in the indoor culture (Fig. 1c), whilst PC was the main PL in the outdoor culture (Fig. 1d). However, it must be noted that PE may be easily converted into PC [28]. The real nature of the change was best understood if we looked at fatty acid content in weight (i.e., mg fatty acid g^{-1} dry biomass). In fact, NLs showed a significant increase from 12 to 16 mg g^{-1} of dry biomass, for indoor and outdoor cultures, respectively. But at the same time, GLs increased from 8 to 47 mg g^{-1}, as well as total fatty acids which shifted from 26 to 80 mg g^{-1} (data not shown). Therefore, the GL increase was not at the expense of NLs, was not a transformation of NLs into GLs, but a net increase of GLs.

Such profound changes in type of lipids are to be expected as a consequence of specific growth conditions [27]. The outdoor culture was taken from a continuous tubular photobioreactor culture, under natural sunlight, with high irradiances in spring and summer in southern Spain and pH controlled by automatic injection of CO_2. The outdoor culture was therefore in nearly optimal growing conditions, so that there was probably a high rate of metabolism producing a large amount of the lipid classes characteristic of young cells, especially MGD and DGD, which are the main chloroplast lipids [29, 30]. The lipid-class composition of this outdoor culture was similar to that reported for the same strain of *P. tricornutum* in logarithmic phase [5]. Similar changes in lipid classes have also been reported in *P. cruentum* as result of changes in temperature and cell concentration [4]. The absence of the two unidentified PLs in the outdoor biomass was also noteworthy. It is likely that these PLs are only produced in older cultures (e.g., at stationary phase).

Fatty acid composition of lipid classes

An array of peaks below 14:0 and between 14:0 and 16:0, were always found in the GC chromatograms. These peaks are probably short-chain fatty acids (e.g., 10:0 and 12:0) or branched short-chain fatty acids [31, 32]; we have collectively called unknown short-chain fatty acids (USCFAs). As individuals, these peaks were mostly irrelevant, but taken together, they represented a significant amount between 4–6% of total fatty acids in the biomass (Tables 1–4). These peaks could be considered artifacts from either non fatty acid methyl esters (FAMEs) or the result of poor laboratory practices. However, we have several arguments to support the non-artifactual nature of these peaks. First, following an extended procedure of saponification [14] to have "bona fide" FAMEs, these array of peaks were also present. Second, they also appeared after GC-analysis of fresh biomass. Finally, if they were artifacts they would be randomly distributed among lipid classes instead of mainly concentrated in NLs (Tables 1–4), as will be discussed below. Although neglecting USCFAs could not invalidate the results for biomass composition, as will be shown below, these USCFAs were quite important in some types of lipids

Heterogeneous fatty acid profiles of the three microalgae studied were obviously reflected in lipid fractions and classes, but despite these obvious differences some generalizations emerge. MAG and DAG were

Table 1. Lipid and fatty acid composition of *Isochrysis galbana*. Values are averages of three independent measurements. Standard errors are shown between parentheses

	Lipid (% of fatty acids)												
	MAG	DAG	TAG	SQD	DGD	MGD	PI	PC	PG	PE	PLX1	PLX2	Biomass
14:0	4.8	4.9	4.8	31.5	10.0	5.8	18.0	6.5	5.3	4.0	6.5	1.7	10.9
	(0.0)	(0.0)	(0.2)	(1.1)	(0.7)	(0.3)	(3.2)	(2.3)	(0.9)	(0.3)	(2.2)	(1.7)	(0.1)
16:0	8.2	8.2	12.3	28.6	15.0	8.2	27.9	22.0	19.4	7.2	11.5	7.5	13.7
	(0.1)	(0.3)	(0.3)	(1.0)	(0.6)	(0.2)	(0.2)	(0.6)	(1.6)	(0.5)	(0.7)	(1.3)	(0.1)
16:1	12.3	11.2	21.7	19.5	18.0	20.41	4.3	9.7	7.8	5.0	10.1	13.7	16.1
	(0.1)	(0.4)	(0.7)	(0.2)	(0.0)	(2.1)	(0.8)	(1.0)	(1.8)	(0.0)	(1.7)	(3.7)	(0.2)
18:0	0.7	0.8	1.2	0.5	0.4	1.1	0.6	0.5	1.1	0.8	1.7	1.2	0.2
	(0.1)	(0.1)	(0.1)	(0.0)	(0.0)	(0.1)	(0.1)	(0.0)	(0.2)	(0.1)	(0.0)	(0.0)	(0.0)
18:1n-9	1.1	1.1	4.9	0.8	0.7	1.5	0.9	1.0	1.5	1.0	6.2	1.5	0.8
	(0.1)	(0.1)	(2.5)	(0.0)	(0.0)	(0.1)	(0.1)	(0.1)	(0.2)	(0.1)	(0.2)	(0.3)	(0.0)
18:1n-7	0.6	1.7	1.2	4.1	1.5	0.9	2.8	1.6	2.8	1.2	2.0	0.9	1.5
	(0.0)	(0.6)	(0.1)	(0.1)	(0.1)	(0.0)	(0.1)	(0.2)	(0.3)	(0.2)	(0.1)	(0.2)	(0.0)
18:2n-6	1.5	1.4	2.2	0.6	1.4	2.2	0.7	0.6	0.2	0.9	8.7	2.1	0.8
	(0.1)	(0.1)	(0.2)	(0.0)	(0.1)	(0.1)	(0.2)	(0.0)	(0.2)	(0.0)	(3.7)	(0.4)	(0.3)
18:3n-3	2.1	1.2	1.5	0.7	2.9	3.9	1.0	0.8	0.4	1.2	1.8	3.8	2.0
	(0.1)	(0.1)	(0.0)	(0.1)	(0.1)	(0.2)	(0.2)	(0.1)	(0.4)	(0.1)	(0.3)	(0.8)	(0.0)
18:4n-3	10.1	6.5	7.2	2.7	12.0	14.7	2.0	2.0	2.7	0.9	6.0	13.7	8.8
	(0.1)	(0.6)	(0.4)	(0.6)	(0.4)	(0.3)	(0.0)	(0.0)	(1.1)	(0.9)	(1.1)	(2.6)	(0.3)
20:5n-3	16.1	12.9	25.6	7.7	25.4	27.8	17.8	38.6	10.9	5.5	18.3	26.5	26.0
	(2.0)	(1.4)	(1.4)	(1.1)	(0.9)	(0.6)	(0.5)	(3.5)	(4.1)	(1.7)	(0.7)	(3.6)	(1.1)
22:5	0.7	0.9	1.2	0.1	0.7	0.6	1.7	2.0	2.7	7.2	6.4	1.0	0.4
	(0.0)	(0.1)	(0.0)	(0.1)	(0.0)	(0.1)	(0.2)	(0.8)	(0.3)	(1.4)	(0.5)	(0.2)	(0.4)
22:6n-3	5.4	5.3	8.1	1.0	7.1	4.7	10.0	11.3	39.0	63.6	14.8	4.8	11.8
	(0.0)	(0.3)	(0.5)	(0.1)	(0.3)	(0.8)	(2.4)	(0.0)	(4.6)	(1.7)	(0.6)	(0.2)	(1.4)
USCFAs	31.5	38.1	4.7	1.1	0.9	4.8	0.9	1.0	2.4	0.8	4.3	19.0	5.0
	(1.4)	(2.7)	(0.4)	(0.3)	(0.1)	(1.4)	(0.1)	(0.0)	(0.4)	(0.7)	(0.6)	(11.7)	(0.0)
Others	4.9	5.8	3.4	1.1	4.0	3.4	1.4	2.4	3.8	0.7	1.7	2.6	2.0

Table 2. Lipid and fatty acid composition of *Porphyridium cruentum*. Values are averages of three independent measurements. Standard errors are shown between parentheses

	Lipid (% of fatty acids)												
	MAG	DAG	TAG	SQD	DGD	MGD	PI	PC	PG	PE	PLX1	PLX2	Biomass
14:0	7.0	6.5	1.6	0.2	0.4	1.7	1.2	0.5	1.6	2.2	0.0	3.4	1.3
	(0.9)	(0.1)	(0.1)	(0.2)	(0.0)	(0.2)	(0.7)	(0.3)	(0.7)	(1.0)	(0.0)	(0.3)	(0.1)
16:0	21.3	19.6	21.1	48.2	40.2	30.3	53.4	42.4	42.1	39.9	62.4	10.4	29.7
	(3.3)	(0.4)	(1.3)	(2.2)	(0.6)	(0.9)	(3.4)	(2.6)	(1.2)	(1.9)	(5.2)	(8.0)	(0.2)
16:1	1.1	0.6	1.5	0.5	2.9	2.1	0.6	2.8	8.9	2.6	4.5	5.6	1.4
	(0.6)	(0.2)	(0.6)	(0.3)	(1.1)	(0.1)	(0.6)	(1.5)	(5.3)	(2.6)	(4.5)	(1.0)	(0.5)
18:0	6.1	1.6	3.7	1.9	2.0	6.6	6.0	2.3	7.0	11.4	16.9	9.7	0.8
	(3.1)	(0.1)	(0.5)	(0.1)	(1.0)	(1.7)	(2.1)	(0.3)	(1.9)	(3.2)	(5.8)	(1.1)	(0.0)
18:1n-9	4.4	1.3	4.0	1.2	1.6	2.5	1.6	2.8	0.0	7.0	0.0	3.8	0.8
	(0.6)	(0.1)	(1.4)	(0.1)	(0.7)	(0.0)	(1.0)	(0.5)	(0.0)	(2.5)	(0.0)	(0.7)	(0.0)
18:1n-7	0.0	0.0	0.9	2.2	2.5	1.3	0.8	3.5	15.8	2.0	0.0	0.0	1.9
	(0.0)	(0.0)	(0.1)	(0.1)	(0.1)	(0.2)	(0.8)	(0.1)	(3.4)	(2.0)	(0.0)	(0.0)	(0.0)
18:2n-6	5.8	7.8	12.2	4.8	6.5	7.8	9.5	6.8	3.2	1.9	0.0	3.7	8.0
	(0.9)	(0.2)	(0.5)	(0.6)	(0.6)	(0.6)	(1.3)	(0.4)	(1.7)	(2.0)	(0.0)	(2.0)	(0.1)
20:2n-6	0.0	0.0	1.0	6.1	0.4	0.5	1.5	0.3	0.0	0.0	0.0	0.0	0.7
	(0.0)	(0.0)	(0.2)	(0.8)	(0.1)	(0.1)	(1.5)	(0.3)	(0.0)	(0.0)	(0.0)	(0.0)	(0.1)
20:3n-6	0.0	0.5	1.1	0.0	0.7	0.8	0.0	1.6	0.0	0.0	0.0	0.0	0.7
	(0.0)	(0.0)	(0.0)	(0.0)	(0.1)	(0.2)	(0.0)	(0.3)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
20:4n-6	13.6	19.2	24.2	14.0	18.4	21.0	10.9	18.8	10.3	16.8	11.7	3.5	25.0
	(3.1)	(0.3)	(0.2)	(1.0)	(0.4)	(1.9)	(2.4)	(2.9)	(2.1)	(3.6)	(6.2)	(1.8)	(0.4)
20:5n-3	9.2	15.9	15.9	19.2	20.3	16.4	10.9	14.2	4.2	4.0	4.5	3.5	20.9
	(1.7)	(0.1)	(1.1)	(1.4)	(1.6)	(2.8)	(1.4)	(1.3)	(2.7)	(2.0)	(4.5)	(1.8)	(0.2)
USCFAs	31.1	26.6	11.9	0.6	1.1	5.0	3.6	1.7	1.9	10.0	0.0	55.3	5.8
	(8.2)	(0.6)	(1.5)	(0.6)	(0.4)	(1.6)	(2.5)	(1.4)	(1.9)	(5.3)	(0.0)	(9.7)	(0.1)
Others	0.4	0.4	0.9	1.1	3.0	4.0	0.0	2.3	5.0	2.2	0.0	1.1	3.0

characteristically composed of USCFAs and generally contained minor amounts of PUFAs (Tables 1–4). For example, MAG and DAG contained ca 58% of USCFAs in the outdoor culture of *P. tricornutum* (Table 4). Unfortunately, these data cannot be compared, because to the best of our knowledge, it is the first time that MAG and DAG have been quantified and their fatty acid composition reported. Analogous studies [5, 7, 33] have not detected these lipid classes or, if detected, they have not been separated [4].

TAG composition was quite different from the other NLs because it always contained large amounts of the most abundant PUFAs (Tables 1–3). For example, even in the outdoor culture of *P. tricornutum*, EPA represented 32% of total TAG fatty acids (Table 4). In other diatoms, such as *Navicula muralis* and *N. incerta*, EPA content in TAG was 21% and 26%, respectively [2, 28]. The fatty acid composition of TAG in the Prymnesiophyte, *P. lutheri* [7], was generally similar to our results for *I. galbana* (Table 1), since the main fatty acids in TAG were 14:0, 16:0, 16:1, EPA and DHA in both Prymnesiophytes. For instance, EPA content in *P. lutheri* TAG varied between 13% and 25% [7] and was 26% in *I. galbana* (Table 1). The *P. cruentum* TAG results (Table 2) were also in agreement with others previously reported [3, 4].

Sulphoquinovose diacylglycerol (SQD) probably has the most characteristic composition of the three microalgae in our study, since 14:0, 16:0, and 16:1 accounted for ca 70% of the total fatty acids in this class and PUFA was significantly depleted (Tables 1–4). This is consistent with similar reports for many other microalgae [3, 5–7, 19, 28, 33]. For example, in *P. lutheri* the sum of 14:0, 16:0, and 16:1 in SQD was 80% of total fatty acids [7]. *Porphyridium cruentum* was again an exception because SQD contained "only" 48% 16:0 and significant amounts of AA and EPA (Table 3), which was in agreement with some published data [3], but disagreed with reported PUFA content [4]. Relatively high EPA content in SQD have also been reported in *Chryptomonas* sp., *N. incerta* [2] and *Skeletonema costatum* [17].

MGD and DGD fatty acid compositions were similar, with high PUFA contents (Tables 1–4). The results for *I. galbana* (Table 1) were quite similar to those of the Prymnesiophyte *P. lutheri*, in which the major MGD fatty acids were 16:1 and EPA [7]. A special feature of these lipid classes in *P. tricornutum* was their high content of C_{16} PUFAs in indoor (Table 3), as well as in outdoor culture (Table 4), which agrees

Table 3. Lipid and fatty acid composition of *Phaeodactylum tricornutum*. Values are averages ot three independent measurements. Standard errors are shown between parentheses

	Lipid (% of fatty acids)												
	MAG	DAG	TAG	SQD	DGD	MGD	PI	PC	PG	PE	PLX1	PLX2	Biomass
14:0	4.7	7.7	4.3	15.2	1.7	1.0	6.2	3.4	3.8	6.2	0.0	1.7	6.3
	(1.4)	(1.6)	(0.1)	(0.9)	(1.0)	(0.3)	(2.5)	(0.8)	(2.1)	(2.3)	(0.0)	(1.7)	(0.0)
16:0	16.4	9.9	13.3	36.9	12.3	6.8	23.7	13.3	20.6	21.1	39.3	56.2	14.4
	(2.7)	(0.6)	(2.8)	(1.8)	(1.6)	(1.5)	(6.2)	(1.9)	(3.8)	(6.0)	(7.4)	(22.0)	(0.6)
16:1	10.0	6.4	17.4	30.6	21.7	19.5	27.7	23.6	36.7	17.3	19.3	10.7	21.6
	(0.6)	(1.2)	(0.7)	(1.2)	(2.5)	(0.8)	(3.0)	(2.9)	(2.5)	(5.4)	(9.7)	(5.8)	(0.5)
16:2n-4	1.6	0.8	4.8	2.8	13.4	13.4	5.2	7.8	3.4	1.7	0.0	0.4	7.0
	(0.8)	(0.5)	(0.9)	(1.4)	(1.7)	(0.7)	(3.0)	(3.3)	(1.8)	(1.1)	(0.0)	(0.4)	(0.1)
16:3n-4	3.3	0.5	2.1	0.0	17.1	18.1	0.0	9.8	2.0	0.5	0.0	0.0	7.0
	(0.7)	(0.3)	(0.9)	(0.0)	(6.1)	(1.8)	(0.0)	(9.2)	(1.0)	(0.5)	(0.0)	(0.0)	(0.1)
16:4n-1	2.4	0.0	0.5	0.0	5.1	3.7	0.0	3.0	0.0	0.4	0.0	0.0	2.2
	(2.0)	(0.0)	(0.1)	(0.0)	(1.9)	(1.2)	(0.0)	(3.0)	(0.0)	(0.4)	(0.0)	(0.0)	(0.0)
18:0	0.6	1.5	2.5	1.5	0.4	1.1	0.6	0.5	3.8	10.8	15.4	3.5	2.5
	(0.6)	(0.2)	(0.4)	(0.8)	(0.2)	(0.6)	(0.6)	(0.3)	(3.0)	(10.8)	(15.4)	(3.5)	(0.8)
18:1n-9	0.9	1.0	1.4	0.0	0.5	0.4	1.0	2.4	1.3	5.3	0.0	0.8	0.7
	(0.9)	(0.5)	(0.2)	(0.0)	(0.3)	(0.2)	(1.0)	(0.9)	(0.7)	(3.6)	(0.0)	(0.8)	(0.0)
18:1n-7	0.0	0.0	0.1	0.0	0.5	0.2	0.6	1.0	1.7	0.0	0.0	0.0	0.7
	(0.0)	(0.0)	(0.1)	(0.0)	(0.3)	(0.1)	(0.6)	(0.5)	(0.9)	(0.0)	(0.0)	(0.0)	(0.0)
18:2n-6	0.8	0.8	1.0	0.0	2.1	0.9	9.5	8.0	3.1	4.1	3.9	0.8	2.3
	(0.8)	(0.4)	(0.2)	(0.0)	(1.5)	(0.5)	(1.2)	(3.0)	(1.6)	(2.1)	(3.9)	(0.7)	(0.0)
20:4n-6	2.1	0.2	3.7	0.7	2.4	2.6	3.8	5.4	2.0	5.1	0.0	0.0	3.3
	(1.1)	(0.2)	(0.4)	(0.6)	(0.9)	(0.1)	(1.9)	(1.7)	(1.0)	(1.6)	(0.0)	(0.0)	(0.1)
20:5n-3	11.6	4.5	35.5	10.7	21.9	30.7	18.7	18.2	21.0	22.4	19.4	16.0	26.0
	(3.6)	(1.7)	(3.0)	(1.6)	(0.9)	(2.5)	(6.2)	(3.2)	(1.0)	(10.3)	(10.5)	(8.2)	(0.8)
22:6n-3	3.3	0.3	1.2	0.0	0.8	0.5	3.0	3.6	0.6	3.6	2.6	0.0	1.7
	(3.3)	(0.3)	(0.9)	(0.0)	(0.6)	(0.4)	(1.5)	(1.3)	(0.6)	(1.9)	(2.6)	(0.0)	(0.1)
USCFAs	37.0	59.5	7.7	0.0	0.1	0.9	0.0	0.0	0.0	1.5	0.0	9.9	4.3
	(3.4)	(3.7)	(1.7)	(0.0)	(0.1)	(0.7)	(0.0)	(0.0)	(0.0)	(1.5)	(0.0)	(5.0)	(0.4)
Others	5.3	6.9	4.5	1.6	0.0	0.2	0.0	0.0	0.0	0.0	0.1	0.0	0.0

with similar studies on this species [5, 33] and other diatoms [2, 28]. *Porphyridium cruentum* had large amounts of EPA and AA in both GLs (Table 3) as previously reported [3], but in contrast to [4] which reported very low AA content.

Phosphatidylinositol (PI) fatty acid composition in the present study was also typical, containing 16:0 and 16:1 (and 14:0 in *I. galbana*), which together accounted for over 50% of the total fatty acids and minor, although significant, contributions of the main PUFAs (Tables 1–3). Few reports include data on PI in microalgae and there is no consistent pattern in the microalgae [2, 3, 6, 28]. Thus, 16:0 and 16:1 comprised 74% of the total fatty acids and traces of EPA were found in *M. subterraneous* [3]. In contrast, two diatoms, *N. muralis* and *N. incerta*, showed a high C_{16} fatty acid content, as well as 16 and 27% of EPA, respectively [2, 28], which agrees with these results for the diatom, *P. tricornutum* (Tables 3 and 4).

PC was essentially composed of the main C_{16} fatty acids (16:0 in *P. cruentum* and *I. galbana*, 16:1 and 16:0 in *P. tricornutum*) and the major PUFAs of the species (EPA in *P. tricornutum*, EPA and DHA in *I. galbana*, and EPA and AA in *P. cruentum*) (Tables 1–3). *Cryptomonas* sp., *Glenodinium* sp., *N. muralis*, *N. incerta* [2], and *Chlorella vulgaris* [34] fitted this description but *Ochromonas danica*, *M. subterraneous* [3], and *Nannochloropsis* [23] did not. Therefore, no consistent pattern could be found for PC in microalgae. The fatty acid composition of PC in *P. tricornutum* (Tables 3 and 4) was generally in agreement with that previously reported for this species [5, 33]. These data for *P. cruentum* were also similar to those of [3], but differed from others [4, 25], who reported very low EPA content. Finally, PC fatty acid composition in *I. galbana* is similar to an Antarctic Prymnesiophtyte which was essentially made up of 16:0 and the major PUFAs, with the peculiarity that 18:5n-3 was also present in this species [19], instead of EPA as in *I. galbana* (Table 1).

Phosphatidylglycerol (PG) fatty acid composition was essentially similar to PC, but with significantly more 16:1 (Tables 1–4). This was especially remarkable in *P. cruentum*, because this fatty acid represented only 1% of average acyl-lipids, but 9% in PG (Table 3). Previous *P. cruentum* [3, 25] and other microalgae [2, 19, 33, 35] studies also reported a specific accumulation of 16:1 in PG. On the other hand it is likely

Table 4. Lipid and fatty acid composition of an outdoor culture of Phaeodactylum tricornutum. Values are averages of three independent measurements. Standard errors are shown between parentheses

	Lipid (% of fatty acid)										
	MAG	DAG	TAG	SQD	DGD	MGD	PI	PC	PG	PE	Biomass
14:0	10.6	10.5	2.7	14.2	2.7	0.6	6.2	2.7	4.8	5.2	6.1
	(2.1)	(0.8)	(0.2)	(0.9)	(0.6)	(0.1)	(0.3)	(0.6)	(0.2)	(0.8)	(0.2)
16:0	5.4	6.6	14.1	32.4	13.5	3.3	26.7	12.9	13.9	12.5	14.1
	(1.9)	(0.6)	(2.2)	(2.2)	(0.5)	(0.4)	(2.6)	(1.1)	(1.5)	(2.2)	(0.7)
16:	15.6	6.5	16.7	29.2	26.4	13.3	28.6	22.1	32.3	22.0	20.1
	(2.9)	(0.6)	(0.4)	(2.2)	(1.6)	(0.5)	(1.4)	(1.0)	(4.7)	(2.0)	(0.4)
16:2n-4	2.5	1.3	5.8	10.0	8.5	8.7	3.2	4.0	3.1	2.1	5.2
	(1.4)	(0.2)	(2.3)	(7.1)	(0.4)	(0.2)	(0.4)	(0.1)	(0.4)	(2.1)	(0.1)
16:3n-4	6.0	2.7	6.7	0.6	4.8	25.9	1.1	0.9	0.4	4.3	9.1
	(2.5)	(0.3)	(0.3)	(0.3)	(0.6)	(0.5)	(1.1)	(0.1)	(0.4)	(4.3)	(0.1)
16:4n-1	0.9	0.7	1.7	0.0	0.8	4.9	0.0	0.0	0.0	0.0	1.9
	(0.5)	(0.1)	(0.1)	(0.0)	(0.1)	(0.1)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)
18:1n-9	0.3	0.5	1.0	0.1	0.7	0.2	2.9	3.0	3.3	2.6	0.7
	(0.3)	(0.1)	(0.1)	(0.1)	(0.2)	(0.0)	(0.2)	(0.2)	(0.6)	(1.3)	(0.0)
18:1n-7	0.7	0.0	0.3	0.2	2.1	0.2	0.8	1.9	6.2	2.6	1.0
	(0.7)	(0.0)	(0.0)	(0.2)	(0.3)	(0.0)	(0.8)	(0.1)	(0.8)	(1.4)	(0.0)
18:2n-6	0.3	1.0	1.4	0.1	2.4	1.0	7.6	9.3	7.9	6.5	2.3
	(0.3)	(0.2)	(0.1)	(0.1)	(0.3)	(0.0)	(0.7)	(0.5)	(1.3)	(3.5)	(0.0)
20:4n-6	0.0	0.2	1.1	0.8	2.2	0.7	1.6	4.6	1.9	2.5	1.9
	(0.0)	(0.1)	(0.1)	(0.5)	(0.9)	(0.3)	(0.8)	(0.2)	(1.0)	(1.2)	(0.3)
20:5n-3	6.6	4.9	32.3	11.8	34.5	40.1	16.1	33.4	24.2	36.1	29.5
	(1.6)	(0.6)	(3.0)	(2.0)	(0.4)	(0.6)	(1.5)	(1.7)	(2.7)	(1.0)	(0.7)
22:6n-3	0.0	0.6	1.4	0.1	1.2	0.5	2.9	3.8	1.7	3.6	1.7
	(0.0)	(0.2)	(0.2)	(0.1)	(0.0)	(0.1)	(0.5)	(0.5)	(1.1)	(1.8)	(0.0)
USCFAs	57.4	58.7	8.1	0.3	0.0	0.1	0.0	0.3	0.0	0.0	4.4
	(6.5)	(1.3)	(2.0)	(0.2)	(0.0)	(0.1)	(0.0)	(0.1)	(0.0)	(0.0)	(0.5)
Others	3.7	5.8	6.7	0.2	0.5	2.3	1.1	0.3	0.0	0.0	2.0

this fatty acid may be 16:1n-3*trans* mixed with 16:1n-7, because some reports clearly identified the presence of the *trans*-isomer specifically in PG [2, 6, 19, 25, 33, 35, 36].

No consistent PE fatty acid pattern could be identified in any of the three microalgae examined in this study. PE was composed mainly of DHA (64%) and was poor in 16:0 in *I. galbana* (Table 1), whilst 16:0 or 16:1 were the major fatty acids and DHA minor in *P. cruentum* and *P. tricornutum* (Tables 2–4). The few papers reporting PE in microalgae are also rather inconsistent [2, 3, 6, 19, 25, 28]. The accumulation of DHA in the PL fraction of *I. galbana* was also found in *Isochrysis* T-ISO [37] and PE in particular accumulated DHA in an Antarctic Prymnesiophyte [19], and in the diatom, *S. costatum* [17]. Therefore, the accumulation of DHA in PLs or specifically in PE, does not seem to be exclusive to Prymnesiophytes. Nyberg and Koskimies-Soininen [25], in a detailed studied on *P. cruentum* PLs, reported that 16:0 and AA accounted for over 50% of the total fatty acids in PE. The present study shows that 16:0 and AA comprised 57% of PE and the portion of EPA in PE was similar in both studies.

In spite of the dramatic changes in lipid composition discussed above, the overall fatty acid profile was similar in indoor and outdoor cultures of *P. tricornutum* (Tables 3 and 4). The fatty acid composition within every lipid class remained virtually unchanged, i.e., they did not seem to be affected by culture age or conditions (for example, compare TAG composition in Tables 3 and 4) with four significant exceptions: MGD, DGD, PC and PE. These types of lipids showed a great increase in EPA content from indoor to outdoor (cf. Tables 3 and 4). For example, EPA in MGD increased from 31 to 40%. Nevertheless, as the specific membrane role of EPA, if any, is unknown it is difficult to advance some suggestion about the meaning of this increase.

The present results may be useful for PUFA purification strategies. Although little has been published about PUFA purification from microalgal biomass, currently there are basically two strategies. First, direct saponification of microalgal biomass followed by PUFA purification [38, 39]; and, second, PUFA purification from a lipid fraction or class previously isolated [40, 41]. Both strategies involve the use of an expensive procedure, such as HPLC. The results of the present work showed that a simpler and cheaper procedure may be followed to purify some PUFAs, taking into account the ability of the urea method to concentrate over one and a half the raw material [39].

For example, DHA which was 63.6% of PE of *I. galbana*, could be concentrated near to 100% by the urea method [39] using PE as raw material.

EXPERIMENTAL

The microalgal, species studied were *Isochrysis galbana* Parke, strain ALII4 [11], *Porphyridium cruentum* Näeg. strain UTEX£161, and *Phaeodactylum tricornutum* Bholin, strain UTEX£640. The growing media used were the Ukeless modified medium [42] for *I. galbana*, the Jones' medium [43] for *P. cruentum*, and Mann and Myers' medium [44] for *P. tricornutum*. All cultures were grown in 5-l flasks under continuous light with aeration at 20°. *P. tricornutum* was also cultured in an external tubular photobioreactor exposed to sunlight with pH controlled by automatic CO_2 injection, but with conditions otherwise similar to above (see Ref. [45] for a full description of the system). Indoor cultures were harvested at stationary phase. The *P. tricornutum* biomass cultured outdoors was taken from a continuously maintained linear-phase culture. The microalgal biomass was lyophilized and stored at −30° under Ar.

Total lipids were extracted from 200 mg of lyophilized biomass [46], dried with N_2 and stored, if required, under an inert atmosphere as stated above. Total lipids were fractionated on a silica gel cartridge [47]. Each fr. was dried in a rotary evaporator, resuspended in 2 ml of $CHCl_3$ and, if necessary, stored at −30° under Ar. Each lipid fr. was subsequently separated into individual lipid classes by one-dimensional TLC on silica gel. Plates were activated in an oven at 120° for 2 h before use. Solvents used were petrol–Et_2O–HOAc (80:20:1) for NLs and $CHCl_3$–MeOH–HOAc–H_2O (170:25:25:6) for both polar lipid frs. [21]. I_2 vapour was used as a general stain. After staining, individual bands were scraped off the plates and analyzed by GC. Samples for fatty acid analyses were prepared for GC by direct transmethylation [48] including 19:0 as int. standard for quantitation. GC conditions have been published elsewhere [49]. The following fatty acids were used as standards: 14:0, 16:0, 16:1n-7, 16:2n-4, 16:3n-4, 16:4n-1, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 18:2n-4, 18:3n-6, 18:3n-4, 18:3n-3, 18:4n-3, 18:4n-1, 20:1n-9, 20:2n-6, 20:3n-6, 20:4n-6, 20:3n-3, 20:4n-3, 20:5n-3, 22:1n-9, 21:5n-3, 22:5n-3 and 22:6n-3.

Lipid fraction purity was determined after TLC by specific staining: α-naphthol for GLs and phosphate stain for PLs [21, 50, 51]. Lipid classes were identified by co-chromatography with authentic standards when commercially available, or by the appropriate use of the following more or less specific stains: α-naphtol (GLs), phosphate stain (PLs), Dragendorff's reagent (PC), ninhydrin (amino-lipids) [21, 51] and cresyl violet (SQD) [50]. Two-dimensional TLC was carried out only to confirm lipid class identification by comparison with others (Eichenberger personal communication, Cohen personal communication). Solvents used were first, $CHCl_3$–MeOH–H_2O (65:25:4) and, second, $CHCl_3$–MeOH–ethylpropylamine–NH_4OH (65:cv:0.5:5) [52]. The complete process, from lipid extraction to TLC separation of lipid classes, was repeated three times per microalgal biomass, and therefore, reported data are the averages of three values.

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