Microbiology, Vol. 70, No. 2, 2001, pp. 158–164. Translated from Mikrobiologiya, Vol. 70, No. 2, 2001, pp. 196–203.

EXPERIMENTAL ARTICLES

Effect of Vitamin E and Its Analogues Having Various Molecular Structures on the Growth and Lipid Composition of *Pythium debaryanum*

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Received March 1, 2000; in final form, September 20, 2000

Abstract—The effect of exogenously added vitamin E and its synthetic analogues (the hydrophilic form of vitamin E and chromans C_{13} and C_1) at a concentration of 9.86×10^{-5} M on the growth, lipogenic activity, and the fatty acid composition of the eicosapolyenoic acid–synthesizing oomycete *Pythium debaryanum* was studied. The effect was found to depend on the molecular structure of particular compounds. For instance, vitamin E and chroman C_{13} stimulated fungal growth, whereas chroman C_1 inhibited it. The hydrophilic form of vitamin E enhanced the lipogenic activity of the oomycete. The studied compounds, which possess antioxidant activity, did not exert any noticeable effect on the content of eicosapolyenoic acids and the degree of the unsaturation of fungal lipids.

Key words: oomycete, lipids, eicosapolyenoic acids, vitamin E, the hydrophilic form of vitamin E, chroman C_{13}, chroman C_1.

The growth, cytodifferentiation, and metabolism of fungi depend to a large extent on environmental conditions. This allows fungal metabolism to be controlled by varying cultivation conditions or adding physiologically active compounds (the so-called biological effectors) to the growth medium. In particular, exogenously added sterols were found to affect the sexual reproduction, growth, and lipogenic activity of oomycetes [1, 2]; in this case, ergosterol (or provitamin D_2) enhanced the cellular level of polyenoic fatty acids in *Pythium debaryanum* [2].

Vitamin E (or α-tocopherol) is known as an antioxidant that inhibits lipid peroxidation [3] and is involved in the regulation of various membrane processes [4, 5]. Theory explains the mechanism of the action of antioxidants by their ability to terminate lipid peroxidation reactions through interaction with the hydroperoxyl radicals of fatty acids. The antioxidant activity of α-tocopherols is most pronounced with respect to polyenoic fatty acids, which are the main substrates of lipid peroxidation reactions [4]. In recent years, the effect of endogenous and exogenous antioxidants on the metabolism of membrane lipids has been extensively studied. Along with their action as antioxidants, the natural and synthetic forms of vitamin E can serve as the effectors of various membrane-bound enzymes, desaturases in particular [4–8].

It should be noted that most of the relevant investigations were carried out in vitro. The investigations performed at the cellular level deal mainly with animal and plant cells [9]. The widest spectrum of organisms was studied by Burlakova *et al.* [10–12]. The studies involving mycromycetes are scarce [13, 14].

To the best of our knowledge, there is no information available on the effect of naturally occurring vitamins and their synthetic analogues on the oomycetes and other fungi capable of synthesizing eicosapolyenoic fatty acids.

For this reason, the present work was aimed at studying the effect of exogenously added vitamin E, its poorly studied synthetic analogues (chroman C_{13} and chroman C_1), and disodium tocopheryl phosphate salt on the growth, lipogenesis, and fatty acid composition of the oomycete *Pythium debaryanum*, which is capable of synthesizing eicosapolyenoic fatty acids. It should be noted that the major eicosapolyenoic acids of this oomycete are arachidonic (5,8,11,14-eicosatetraenoic, $C_{20:4}$) and 5,8,11,14,17-eicosapentaenoic ($C_{20:5}$) acids, whereas dihomo-γ-linolenic (8,11,14-eicosatrienoic, $C_{20:3}$) acid is present in minor amounts (within 0.39–1.31% of the total content of cellular fatty acids).

MATERIALS AND METHODS

Experiments were carried out with the oomycete *Pythium debaryanum*, a representative of the genus *Pythium*, whose members parasitize different crops and affect the forest tree roots [15]. *P. debaryanum* attracts

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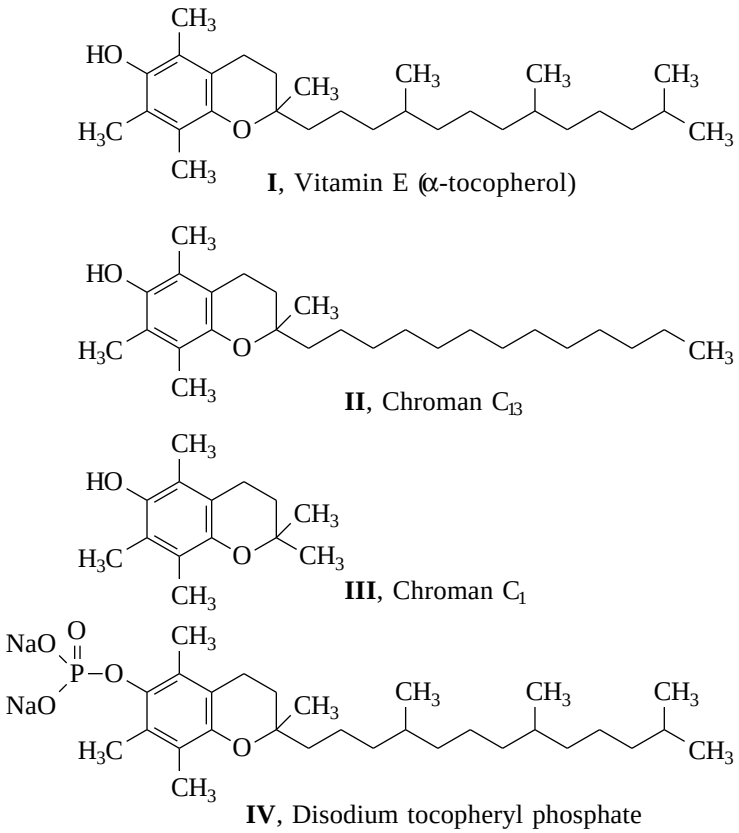

I, Vitamin E (α-tocopherol)

II, Chroman C_{13}

III, Chroman C_1

IV, Disodium tocopheryl phosphate

Fig. 1. Molecular structures of vitamin E and its analogues.

the interest of researchers as a fungus-synthesizing, pharmacologically active lipid [16].

P. debaryanum was cultivated in 250-ml flasks with 50 ml of a medium containing (%) glucose, 4; protein hydrolysate Belkazin, 2; yeast extract, 0.1; $MgSO_4 \cdot 7H_2O$, 0.025; and KH_2PO_4, 0.14; in tap water. The medium was inoculated, in an amount of 2–5%, with cells washed off of wort agar. Cultivation was performed in a submerged mode at 26–28°C on a shaker (220 rpm).

The accumulation of the biomass, its lipid content, and the fatty acid composition were determined throughout four days of the fungus cultivation. To estimate the dry weight of the biomass, the culture was filtered through a cloth, and the residue was dried to a constant weight at 95°C.

Lipids were analyzed after extracting them from the biomass by the Bligh and Dyer method [17]. Lipid content was expressed as a percentage of the dry biomass. Fatty acids were analyzed after their conversion into methyl esters [17]. The methyl esters were quantified on a gas–liquid chromatograph (model 3700) equipped with a flame ionization detector and a (1 m × 3 mm ID) glass column packed with Chromosorb WAW-DMCS-HP (80–100 mesh) containing 17% diethylglycol succinate. The carrier gas was argon at a flow rate of 50 ml/min. The temperatures of the column and evaporator were held at 180 and 250°C, respectively. The fatty acid methyl esters were identified by comparing their retention times with those of the reference samples and quantified by the triangular method [17].

In experiments, we used vitamin E (*dl*-α-tocopherol) (Fig. 1, compound **I**) and its analogues possessing antioxidant activity: chroman C_{13} (2,5,7,8-tetramethyl-2-tridecyl-chroman-6-ol) (Fig. 1, compound **II**), chroman C_1 (2,2,5,7,8-pentamethyl-chroman-6-ol) (Fig. 1, compound **III**), and the water-soluble form of vitamin E (the disodium tocopheryl phosphate salt) (Fig. 1, compound **IV**). All these substances were synthesized in the Department of Chemistry and Technology of Organic Compounds of the Moscow State Academy of Fine Chemical Technology.

Disodium tocopheryl phosphate was added to the medium as an aqueous solution, whereas vitamin E and the chromans, which are insoluble in water, were added as ethanol solutions. In this case, as special experiments showed, the concentration of the ethanol introduced into the medium with the solutions (0.3 vol%) did not inhibit the oomycete growth, virtually did not influence the accumulation of lipids in the mycelium, and insignificantly increased the relative content of polyenoic acids (PEA), including eicosapolyenoic acids (EPEA). In all the experiments performed, an equivalent amount of ethanol (0.3 vol%) was added to the control flasks.

Table 1. Effect of vitamin E on the growth and lipogenesis of the oomycete *P. debaryanum*

Parameter	Control		Vitamin E	
Cultivation time, h	72	96	72	96
Fatty acids, % of total				
14 : 0	5.85	4.97	6.17	5.05
16 : 0	10.96	11.47	12.76	11.14
16 : 1	1.06	1.16	11.11	1.07
18 : 0	5.97	6.17	5.27	6.55
18 : 1	32.91	37.49	37.89	32.70
18 : 2	14.06	12.64	13.34	13.51
18 : 3	2.07	1.71	1.52	3.02
20 : 0	7.09	7.73	5.68	7.91
20 : 3	0.68	0.80	0.45	0.94
20 : 4	11.40	9.40	9.55	10.80
20 : 5	8.05	6.46	6.26	7.31
Degree of unsaturation*	2.35	2.30	2.35	2.26
PEA, %	36.26	31.02	31.12	35.58
EPEA, %	20.13	16.66	16.26	19.05
EPEA, mg/l	146.24	200.59	155.90	307.69

* The degree of unsaturation was calculated as the ratio of the content of unsaturated fatty acids to the content of saturated fatty acids.

Table 2. Effect of chromans C_{13} and C_1 on the growth and lipogenesis of the oomycete *P. debaryanum*

Parameter	Control		Chroman C_{13}		Chroman C_1	
Cultivation time, h	72	96	72	96	72	96
Fatty acids, % of total						
14 : 0	5.71	7.82	5.23	6.18	5.19	3.75
16 : 0	14.47	15.34	18.14	15.59	14.27	20.40
16 : 1	1.29	1.15	2.25	2.24	1.65	2.13
18 : 0	4.38	4.99	3.67	4.52	3.03	2.41
18 : 1	33.37	35.31	30.32	32.02	30.07	23.10
18 : 2	15.69	13.60	15.69	14.13	18.02	20.18
18 : 3	2.15	1.94	1.96	2.32	1.81	2.55
20 : 0	4.36	5.53	4.66	5.26	4.15	4.36
20 : 3	0.79	0.79	0.54	0.56	0.72	0.61
20 : 4	9.28	7.15	8.15	7.97	9.84	8.03
20 : 5	8.51	6.38	9.39	9.21	11.25	12.48
Degree of unsaturation	2.46	1.97	2.15	2.17	2.75	2.23
PEA, %	36.42	29.86	35.73	34.19	41.63	43.85
EPEA, %	18.58	14.32	18.08	17.74	21.81	21.12
EPEA, mg/l	179.27	171.84	104.37	202.79	88.58	86.17

In choosing the appropriate concentration of vitamin E and its analogues in our experiments, we took into account the relevant experimental data available in the literature, which indicate that (1) the antioxidant activity of these compounds with respect to polyenoic fatty acids is most pronounced when the antioxidant-to-PEA molar ratio is about 1 : 100 [18]; (2) the effective concentration of natural antioxidants is about 0.3% of the lipid concentration [19]; and (3) the physiological concentration of antioxidants ranges from 10^{-4} to 10^{-7} M [20].

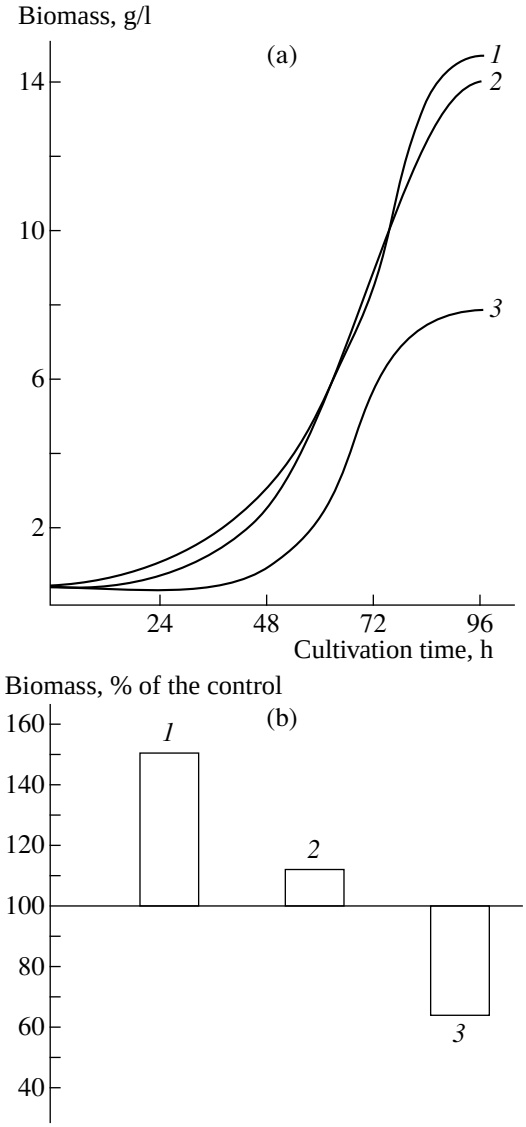

Fig. 2. Effect of vitamin E and its analogues on (a) growth and (b) maximum biomass of the oomycete *P. debaryanum*: *1*, growth and biomass in the presence of vitamin E; *2*, growth and biomass in the presence of chroman C_{13}; and *3*, growth and biomass in the presence of chroman C_1.

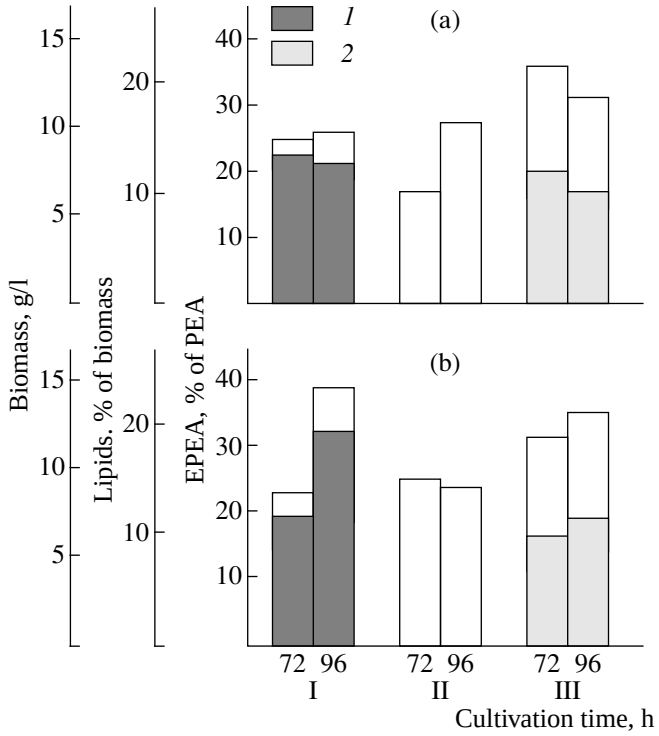

Fig. 3. (I) Biomass, (II) total lipids, and (III) polyenoic acids of the oomycete *P. debaryanum* grown (b) with and (a) without vitamin E. Here and in the legends to Figs. 4 and 5, *1* represents the biomass minus lipids and *2* represents the percentage of eicosapolyenoic acids in the total polyenoic acids.

In the final analysis, with allowance for the data of Solov'eva *et al.* [16] on the average contents of lipids, total fatty acids, and polyenoic fatty acids in the mycelium of *P. debaryanum*, the concentrations of vitamin E and its analogues in the below experiments were chosen to be 9.86×10^{-5}M.

RESULTS AND DISCUSSION

The results presented in Tables 1 and 2 and in Figs. 2–4 show that vitamin E appreciably increased the maximum biomass of the oomycete and caused a delay in its exponential growth (Fig. 2). In the presence of vitamin E, the maximum yield of the biomass (14.7 g/l) exceeded the total and lipid-free biomasses in the control by 51.6 and 54.3%, respectively. At the same time, vitamin E virtually did not affect the lipid content of the biomass: the maximum contents of lipids in the mycelium grown in the presence and in the absence of vitamin E were 15.04 and 16.55%, respectively (Fig. 3). Unlike the oomycete growth in the absence of vitamin E, its growth in the presence of vitamin E continued for as long as 96 h, so that its biomass increased from 8.5 g/l in the 72nd hour of cultivation to 14.7 g/l in the 96th hour. In this case, the lipid content of the mycelium did not noticeably decrease. Earlier, the decrease in the lipid content of the oomycete *P. debaryanum* was explained by the consumption of lipids (predominantly, triacylglycerides) for its energy purposes [16].

The relative content of eicosapolyenoic fatty acids almost did not change: the fatty acids of the mycelia grown with and without vitamin E contained 19.05 and 20.13% of EPEA, respectively. In both cases, the mycelia taken from the stationary growth phase contained greater amounts of lipids than those taken from the phase of active fungal growth (Fig. 3).

The stimulatory effect of vitamin E on the growth of *P. debaryanum* against the background of its insignificant influence on the relative content of polyenoic fatty acids in the mycelium led to a considerable increase in

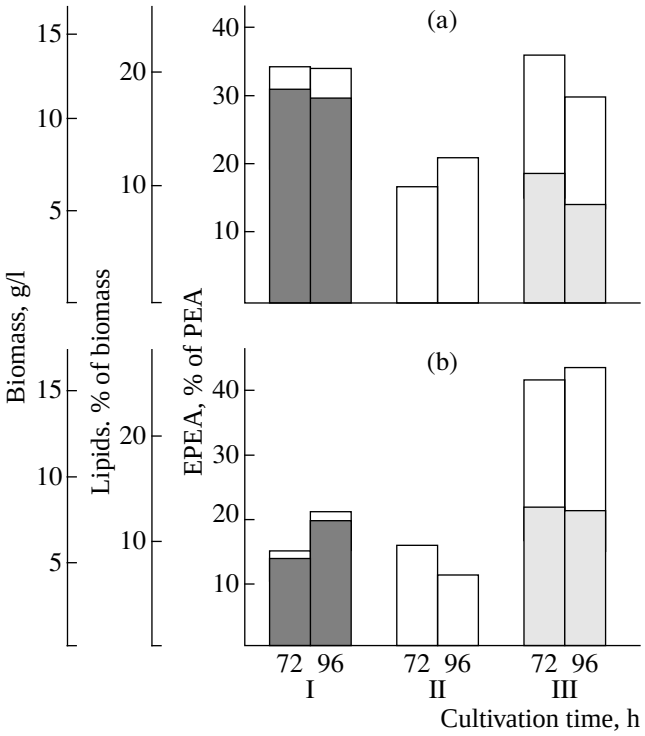

Fig. 4. (I) Biomass, (II) total lipids, and (III) polyenoic acids of the oomycete *P. debaryanum* grown (b) with and (a) without chroman C_1.

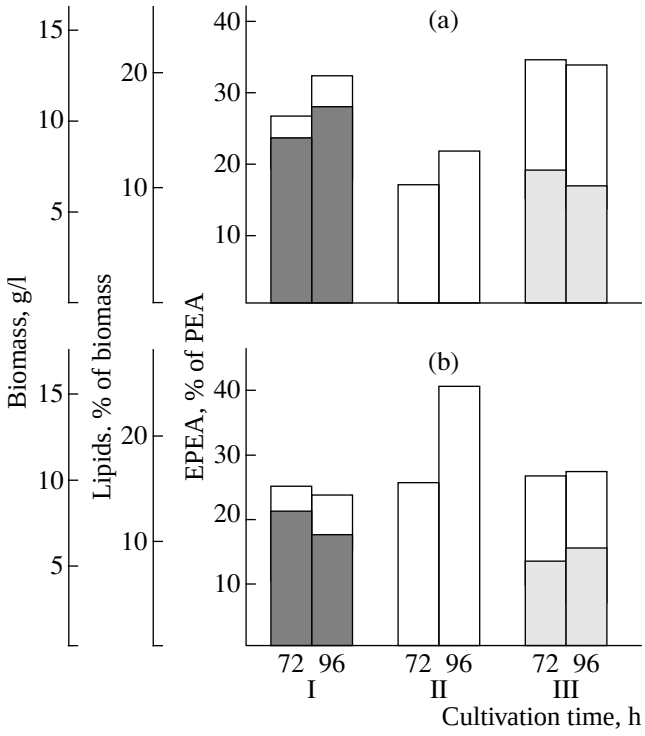

Fig. 5. (I) Biomass, (II) total lipids, and (III) polyenoic acids of the oomycete *P. debaryanum* grown (b) with and (a) without disodium tocopheryl phosphate.

the yield of all polyenoic acids per unit culture volume. Namely, vitamin E stimulated the yield (per unit volume of 96-h culture) of total polyenoic acids from 373.4 to 574.7 mg/l (i.e., by 53.9%), of octadecapolyenoic acids from 172.8 to 266.9 mg/l, and of eicosapolyenoic acids from 200.6 to 307.7 mg/l (Table 1).

It was also found that chroman C_{13} somewhat inhibited the growth of *P. debaryanum* within the first 12 h of cultivation, however, in the course of further cultivation, the effect of chroman C_{13} was like that of vitamin E (Fig. 2). The maximum biomass in the presence of chroman C_{13} was 14.1 g/l, i.e., 12.8% higher than in the control mycelium. Like vitamin E, chroman C_{13} did not considerably affect the lipogenic activity of the oomycete: the total content of lipids in the mycelium grown in the presence of this vitamin E analogue was 10.81% as compared to 12.8% in the control mycelium. After 72 h of cultivation, the content of the eicosapolyenoic acids in the total fatty acids of the mycelium grown in the presence of chroman C_{13} was 18.08% as compared to 18.58% in the control mycelium and to 19.05% in the mycelium grown in the presence of vitamin E. Within 72–96 h of cultivation, the dynamics of the biomass, total lipids, and fatty acids in the presence of chroman C_{13} and vitamin E were almost the same (Fig. 3).

Unlike vitamin E and chroman C_{13}, chroman C_1considerably retarded the growth of the oomycete and decreased its biomass (Fig. 2): the biomass accumulated in the presence of chroman C_1 within 72 h of growth comprised 45% of that in the control mycelium, whereas the maximum biomass accumulated in the presence of this vitamin E analogue made up 64% of the control biomass and 56.74% of the biomass accumulated in the presence of chroman C_{13}. During the phase of active growth, chroman C_1 reduced the total lipid content of the mycelium from 9.5 to 6.8% (Fig. 4). The relative content of polyenoic fatty acids, including eicosapolyenoic acids, in the presence of chroman C_1 was higher than in the control mycelium. Nevertheless, due to the strong inhibition of the oomycete growth, the yield of PEAs and EPEAs per unit culture volume in the presence of chroman C_1 considerably decreased, making up, in the 96-h culture, 178.91 mg/l (the total polyenoic acids), 92.7 mg/l (octadecapolyenoic acids), and 86.2 mg/l (eicosapolyenoic acids) (Table 2).

Disodium tocopheryl phosphate, the water-soluble form of vitamin E, decreased the biomass but increased the lipid content of the mycelium (Table 3 and Fig. 5). After 96 h of cultivation, the lipid content of the mycelium grown in the presence of disodium tocopheryl phosphate reached 24.8%, i.e., almost twofold higher than in the control mycelium and in the mycelium grown in the presence of other α-tocopherols. Reportedly, the increase in the total lipid pool of cells is accompanied by a decrease in the relative content of all and particular polyenoic acids. In the experiments described, however, the percentage of eicosapolyenoic

Table 3. Effect of disodium tocopheryl phosphate on the growth and lipogenesis of the oomycete *P. debaryanum*

Parameter	Control		Disodium tocopheryl phosphate	
Cultivation time, h	72	96	72	96
Fatty acids, % of total				
14 : 0	5.60	5.85	5.19	5.71
16 : 0	12.75	11.76	13.40	9.75
16 : 1	1.13	0.90	1.45	1.10
18 : 0	5.27	6.53	8.60	9.93
18 : 1	32.92	33.12	38.51	39.41
18 : 2	14.56	14.15	11.40	10.81
18 : 3	2.40	3.20	2.08	1.59
20 : 0	6.64	7.59	5.89	6.60
20 : 3	1.03	1.12	0.51	0.39
20 : 4	9.56	9.20	7.74	9.20
20 : 5	8.14	6.58	5.23	5.51
Degree of unsaturation	2.30	2.15	2.02	2.12
PEA, %	35.72	34.25	26.96	27.50
EPEA, %	18.73	16.90	13.48	15.10
EPEA, mg/l	152.21	210.41	149.35	256.10

acids not only failed to decrease but even rose from 13.48 to 15.10%. This fact can be explained by the redistribution of eicosapolyenoic fatty acids between the fractions of polar and neutral lipids.

Thus, it was found that vitamin E and chroman C_{13} stimulate the growth of *P. debaryanum*, whereas chroman C_1 inhibits it. It seems reasonable to relate the inhibitory effect of chroman C_1 on fungal growth to the absence of the side phytol group in its molecule. Indeed, as was suggested by Burlakova *et al.* [10], the phytol group of α-tocopherols serves to fix their molecules in lipids, whose regulatory role in living cells is well known.

It was also found that the hydrophilic form of vitamin E stimulates the lipogenic activity of the oomycete *P. debaryanum* and increases almost twofold the cellular content of pharmacologically active eicosapolyenoic fatty acids.

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