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Research Article

Changes of lipid composition from culture fluid during the production of antibiotic salinomycin

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Abstract

A major step in the biotechnological process of production of antibiotic salinomycin is fermentation, which has the greatest impact on the quality of the final product. Vegetable oils (rapeseed or soybean) are used as nutrient medium during fermentation, with some of the lipids embedded in the final product. Data on their effect on product quality is missing, which is why a study of the changes in the composition of lipids in cultural fluid during the fermentation process was conducted. The composition of the residual lipids isolated from three samples of the culture fluid during fermentation (213, 405 and 490 hours), was investigated before drying the final product. The lipid content of the samples was 6.3%, 3.5% and 6.2%. The main trend is an increase in the amount of saturated fatty acids at the expense of a decrease in unsaturated acids. The amount of tocopherols during the fermentation process increased as well as γ -tocopherol, while the amount of α -tocopherol decreased. The content of carotenoids decreased twice during fermentation. Phosphatidylcholine and phosphatidylethanolamine were identified as major polar lipid components. The main phospholipid classes contain mainly palmitic, stearic and oleic acids. Significant changes in general lipid composition were observed during the biosynthesis process.

Keywords

salinomycin, lipids, fatty acids, tocopherols, carotenoids, phospholipids

Abbreviations

MUFA – Monounsaturated fatty acids; PUFA – Polyunsaturated fatty acids; SFA – Saturated fatty acids; UFA – Unsaturated fatty acids

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Introduction

Salinomycin is an antimicrobial antibiotic that is widely used in veterinary medicine. It has an anti-coccidial effect against all types of coccidiosis in birds and other animals. Salinomycin is active against gram-positive bacteria including mycobacteria and some filamentous fungi. No activity against gram-negative bacteria or yeast has been observed. The acute toxicity of salinomycin in mice was also studied and LD50 was 18 mg/kg intraperitoneally and 50 mg/kg orally (Miyazaki et al. 1974). Salinomycin was effective in reducing chicken mortality and increasing the average body weight of treated infected chickens compared to those of untreated infected controls (Huczyński et al. 2012). Similarly, salinomycin feeding in a group of birds yielded less disease than oocysts and had fewer intestinal lesions that are indicative of intestinal health. Undamaged intestines are crucial for the proper absorption and use of nutrients, which leads to optimal health and productivity (Abdelrahman et al. 2014; Migaki and Babcock 1979; Chappel 1979). In addition, salinomycin has a killing effect on malaria parasites (Mehlhorn et al. 1984).

It is an antibiotic with coccidiostat effect and has shown anticancer efficacy against various cancer cells with selectivity in targeting cancer stem cells (Tyagi and Patro 2019; Versini et al. 2018).

The main stages of any biotechnological production are: 1. Pre-fermentation stage - acceptance and storage of raw materials, preparation and sterilization of the nutrient medium and preparing the culture; 2. Fermentation - the stage at which the product is obtained; 3. Post-fermentation stage - isolation, purification, concentration, drying, grinding and packaging of the product (Volova 1999).

Fig. 1 shows the technological stages of the production of salinomycin in "Biovet AD", Peshtera, Bulgaria.

A major step in the biotechnological process is fermentation, which has the greatest impact on the quality of the final product. During the fermentation two processes are running: the process of decomposition of substances from the external environment, and the process of forming intracellular substances. During fermentation, basic components of the medium are added once or repeatedly. The latter increases the efficiency of the targeted metabolism of microorganisms.

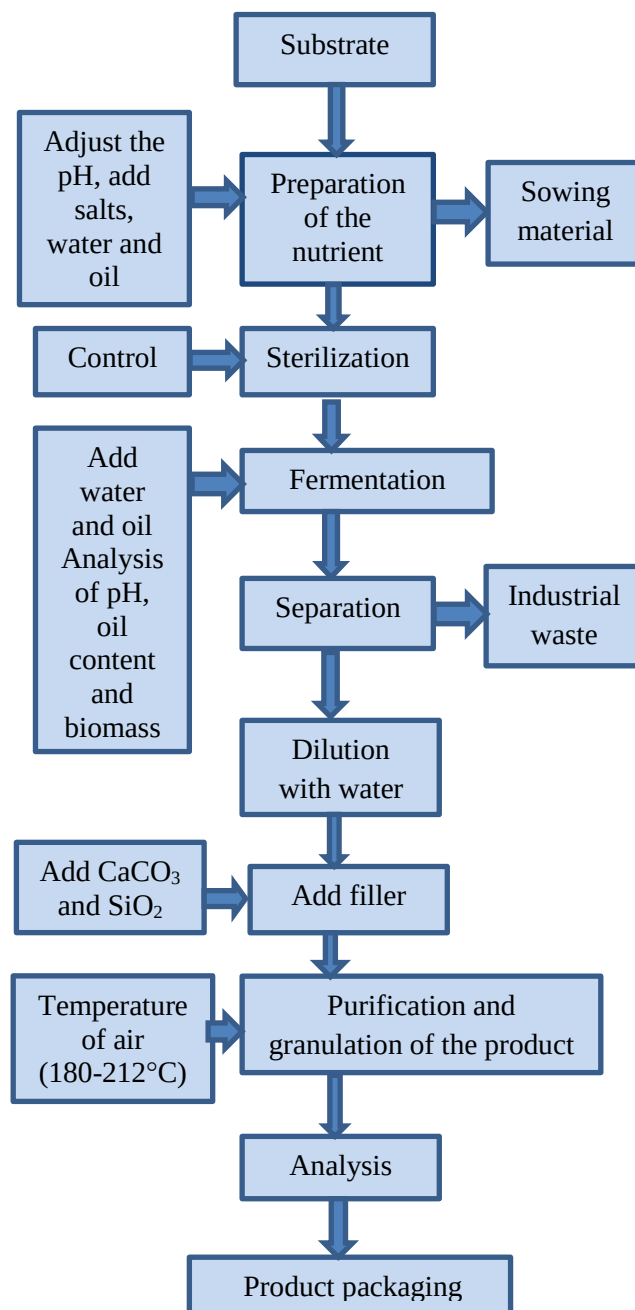


Figure 1. Technological scheme for production of salinomycin

Vegetable oils (most often rapeseed) are used as the nutrient medium during fermentation and some of the lipids are included in the final product. The formed particles from polyether antibiotics include glyceride oils, free fatty acids, and phospholipids such as lecithin. Sometimes, during transport, the individual salinomycin particles form agglomerates,

that affect fluidity of the final product, so it is necessary to determine the content and composition of these lipids. In our previous work the composition of residual lipids isolated from three salinomycin samples with different fluidity, namely good (80%), bad (<80%) and very good (>80%) was investigated (Chuchkov and Antova 2019). In the literature, no data were found about the changes that occur in the composition of lipids during the fermentation process when obtaining the antibiotic salinomycin. There are also no data on the impact of residual lipids in salinomycin particles on product quality. Therefore, the aim of this work was to determine the composition of lipids isolated from intermediate samples in the process of biosynthesis of antibiotic salinomycin and to monitor the changes in their composition during the fermentation process.

Materials and Methods

Samples. Culture fluids taken during fermentation were studied in the process of obtaining salinomycin product in "Biovet AD", Peshtera, Bulgaria. The samples were named according to the time elapsed from the biological process: 213 h, 405 h and after the treatment there was a sample at the end of the process (490 h).

Isolation and quantification of lipid classes. The residual lipids in the studied samples were extracted with hexane. About 60 g of product were taken and 100 mL of hexane were added, then the sample was shaken for 1 hour and this process was repeated three times. The hexane was then evaporated and the lipids were determined gravimetrically (Company standard "Biovet" Peshtera QCD-FPC-0878-09 2008. Amendment: 2014).

Isolation and quantification of lipid classes was carried out by preparative silica gel thin layer chromatography (TLC). The main lipid classes were identified by applying 1 mL of the residual lipids solution in hexane on a 20 cm x 20 cm glass plate with ca 1 mm thick silica gel 60 G layer and developed with hexane: diethyl ether: acetic acid, 80: 20: 1 (v/ v/ v) (Kates 1972). The mixture of lipid classes contained equal parts of docosane, cholesteryl oleate, triolein, cholesterol, 1,3-diolein, 1-monoolein rac-glycerol (Acros organics) and oleic acid (Sigma) used as reference. Triacylglycerols, sterols, diacylglycerols, monoacylglycerols, free fatty acids, hydrocarbons

and waxes were identified and visualized in TLC chamber with iodine crystals and then isolated by elution with diethyl ether. The solvent was evaporated under a stream of nitrogen and the residues were dried to a constant weight.

Fatty acid methyl esters. Fatty acid composition of oils and main classes of phospholipids was determined by gas chromatography (GC) after transesterification the corresponding sample with 2% H₂SO₄ in CH₃OH at 50°C (ISO 12966-2:2011). Fatty acid methyl esters (FAME) were purified by TLC. GC was performed on an HP 5890 series II gas chromatograph unit equipped with a 75 m x 0.25 mm (I.D.) x 18 µm (film thickness) capillary Supelco column and a flame ionization detector. The column temperature was programmed from 140°C (5 min) at 4°C.min⁻¹ to 240°C (3 min); the temperature of the injector and the detector were kept at 250°C. The identification of fatty acids was performed by comparison of retention times of their methyl esters with those of a standard mixture of fatty acids methyl esters subjected to GC under identical experimental conditions (ISO 12966-1:2014). The analytical standard of fatty acids methyl esters was purchased from Supelco. All solvents and reagents were of analytical grade from Merck and were used without additional purification.

Tocopherols. Tocopherols were determined in the oils by high performance liquid chromatography (HPLC) using a fluorescence detector at 290 nm for excitation and 330 nm for emission. It was operated with a Merk-Hitachi liquid chromatograph equipped with a 250 x 4 mm chromatographic column. The stationary phase was Nucleosel C 50-5. The mobile phase was a mixture of hexane : dioxane = 96 : 4 (v/ v) and the flow was 1.5 mL.min⁻¹, a pressure of 50 bar. One percent solution of lipid dissolved in hexane was prepared and 20 µL were injected (ISO 9936:2016). The chromatogram of this solution was compared to that of a standard solution with mixture of pure tocopherols isomers of the exact concentration. The tocopherol content was calculated on the basis of tocopherol peak areas in the sample and tocopherol peak area obtained for the α-tocopherol solution standard and the concentration (µg.mL⁻¹) of α-tocopherol in the working solution.

Carotenoids. One g of the oil was diluted with 20 mL hexane. The absorption was measured using a spectrophotometer at a wavelength of 452 nm (Biehler et al. 2010).

To calculate the carotenoid content (mg.100g⁻¹), the following equation was used:

$$\text{Carotenoids} = \frac{A_{1cm}^{1\%}}{2650} \cdot 10^5, \text{mg/100g}, \quad (1)$$

where

$A_{1cm}^{1\%}$ - absorption at 452 nm of 1% oil solution,
2650 - absorption coefficients of carotenoids.

Phospholipids. The phospholipids from the culture samples were extracted with Folch's procedure. 100 mL of Folch's solution (chloroform: methanol - 2: 1) was added to 30 g of product and stirred for 1 hour; the extraction is repeated three times (Folch et al. 1957). The phospholipids were purified from undesired components by soaking with 20 mL of cooled acetone while the solution was allowed to stand for about 1 hour at -18°C (under these conditions, the phospholipids precipitate and the impurities remain dissolved in acetone and decanted). The phospholipids classes were isolated by a variety of the two-dimensional TLC on 20 x 20 cm glass plates with 0.2 mm Silica gel 60 G layer impregnated with aqueous (NH₄)₂SO₄ (1g in 100 mL water). In the first direction the plate was developed with chloroform : methanol : ammonia, 65 : 25 : 5 (by volume) and in the second – with chloroform : acetone : methanol : acetic acid : water, 50 : 20 : 10 : 10 : 5 (by volume) (Schneider and Daum 2006). The individual phospholipids were identified by comparing the respective R_f values with those of authentic commercial standards subjected to silica gel TLC under identical experimental conditions. The individual phospholipids were visualised in TLC chamber containing crystals of iodine. The quantification was carried out spectrophotometrically against a standard curve by measuring the phosphorous content at 700 nm after scrapping the respective phospholipid spot and mineralization of the substance with a mixture of perchloric acid and sulphuric acid, 1:1 by volume (ISO 10540-1 2014).

Statistical analysis. All measurements were performed in triplicate (n = 3) and the results were presented as mean value with the corresponding standard deviation (SD). Significant differences

were determined by an analysis of variance (Duncan test) with a significance level $p < 0.05$ using IBM SPSS Statistics 19.

Results and Discussion

Three samples of the culture fluid during fermentation from 213 h, 405 h and 490 h, were investigated before drying the final product. The residual lipids isolated from the different samples were 6.3±0.2%, 3.5±0.3% and 6.2±0.2%, respectively. The lipid composition of residual lipids isolated from the three samples of the culture fluid during fermentation (213 h, 405 h and 490 h) is shown in Table 1.

Table 1. Lipid composition of residual lipids isolated from three salinomycin samples during biosynthesis process (213 h, 405 h and 490 h)

Component, %	213 h	405 h	490 h
Monoacyl-glycerols	15.7±0.5 ^b	24.5±0.4 ^a	21.9±0.5 ^c
Diacylglycerols	28.4±0.2 ^c	10.2±0.3 ^a	12.6±0.8 ^b
Triacylglycerols	11.9±0.5 ^b	31.5±1.2 ^c	2.3±0.1 ^a
Free fatty acids	32.1±1.0 ^c	23.0±0.5 ^a	19.0±0.7 ^b
Hydrocarbons and waxes	11.9±0.6 ^b	10.8±0.2 ^a	44.2±0.5 ^c

Values are means ± SD (n = 3). Different letters in the same row mean significant differences ($p < 0.05$) using Duncan test.

The lipid composition of rapeseed oil which had been used for the nutrient medium in the preparation of salinomycin was: triacylglycerols - 96.0%, unsaponifiables - 1.7%, phospholipids and other polar compounds - 1.2%, free fatty acids - 0.5% and small amounts of mono- and diacylglycerols (about 0.5%). The content of monoacylglycerols, diacylglycerols, free fatty acids and hydrocarbons increased while this of triacylglycerols significantly reduced during the biosynthesis process (Table 1). The fatty acid composition, tocopherols composition, the contents of the carotenoids and content and composition of the phospholipids of the samples from the culture fluid were determined. The changes in the fatty acid composition of residual

Table 2. Fatty acids composition of residual lipids isolated from three salinomycin samples during biosynthesis process (213 h, 405 h and 490 h)

Fatty acids, %	Rapeseed oil	213 h	405 h	490 h
C 8:0	–*	–	–	2.5±0.2 ^a
C 10:0	0.1±0.0 ^a	–	–	0.5±0.1 ^b
C 11:0	–	–	–	0.2±0.05
C 14:0	0.9±0.2 ^b	0.1±0.0 ^b	–	0.3±0.1 ^a
C 14:1	0.1±0.0 ^c	0.9±0.1 ^c	0.7±0.1 ^b	0.4±0.1 ^a
C 15:0	–	0.2±0.05 ^a	0.3±0.1 ^a	0.2±0.05 ^a
C 15:1	–	2.0±0.2 ^b	2.2±0.4 ^b	0.2±0.1 ^a
C 16:0	10.3±0.4 ^b	6.4±0.3 ^a	10.6±0.5 ^b	14.4±0.4 ^c
C 16:1	0.4±0.1 ^b	0.2±0.05 ^a	0.1±0.0 ^c	0.2±0.05 ^a
C 17:0	0.4±0.1 ^b	0.3±0.1 ^{ab}	0.4±0.1 ^b	0.2±0.05 ^a
C 17:1	–	0.1±0.0 ^a	0.1±0.05 ^a	0.1±0.05 ^a
C 18:0	3.7±0.2 ^a	5.6±0.3 ^b	18.4±0.5 ^d	10.5±0.7 ^c
C 18:1	67.5±1.0 ^c	77.5±1.2 ^d	48.5±0.8 ^b	46.2±0.5 ^a
C 18:2	13.4±0.4 ^b	0.7±0.1 ^a	14.2±0.2 ^c	21.4±0.5 ^d
C 18:3	2.5±0.1 ^c	–	0.4±0.1 ^a	0.9±0.2 ^b
C 20:0	0.7±0.1 ^a	2.1±0.3 ^b	2.2±0.2 ^b	0.8±0.1 ^a
C 20:1	–	3.9±0.4 ^c	1.9±0.2 ^b	1.0±0.2 ^a

Values are means ± SD ($n = 3$). Different letters in the same row mean significant differences ($p < 0.05$) using Duncan test.

C_{8:0}– caprylic, C_{10:0}– capric, C_{11:0}– undecanoic, C_{14:0}– myristic, C_{14:1}– myristoleic, C_{15:0}– pentadecanoic, C_{15:1}– pentadecenoic, C_{16:0}– palmitic, C_{16:1}– palmitoleic, C_{17:0}– margaric, C_{17:1}– heptadecenoic, C_{18:0}– stearic, C_{18:1}– oleic, C_{18:2}– linoleic, C_{18:3}– linolenic, C_{20:0}– arachidic, C_{20:1}– gadoleic acid.

*– not detected

lipids isolated from three culture fluids of salinomycin samples (213 h, 405 h and 490 h) are given in Table 2. Oleic acid (67.5%) and linoleic acid (13.4%), followed by palmitic acid (10.3%) and stearic acid (3.7%), were found to predominate in the fatty acid composition of the rapeseed oil. Oleic acid was in the highest amount in all samples (in the sample from 213 h it was 77.5%, from 405 h - 48.5% and 490 h - 46.2%, respectively). The amount of oleic acid in the oils isolated from the samples decreased significantly at the expense of an increase in the content of linoleic acid (from 0.7% (213 h) to 21.4% (490 h) and of palmitic acid (from 6.4% (213 h) to 14.4% (490 h)). The residual lipids from the different samples contained higher content of stearic acid (5.6%, 18.4% and 10.5% respectively) compared to that of the source rapeseed oil (3.7%).

Fig. 2 shows the ratio of unsaturated and saturated fatty acids in the rape seed oil and residual lipids isolated from the three salinomycin samples (213 h, 405 h and 490 h).

It was observed that the content of saturated (14.7% to 31.9%) and polyunsaturated fatty acids (0.7% to 22.3%) increased at the expense of a decrease of monounsaturated fatty acids (84.6% to 48.1%) during the fermentation process.

Compared to the composition of the rapeseed oil, there was an increase in the saturated fatty acids (from 16.0 to 29.6%) and a significant decrease in the content of the monounsaturated fatty acids from 68.0 to 48.1%. The amount of polyunsaturated fatty acids during the fermentation process drastically decreased then gradually increased towards the end of the process (from 16.0 to 0.7% in 213 h of the process and reaches 22.3% at the end of the fermentation).

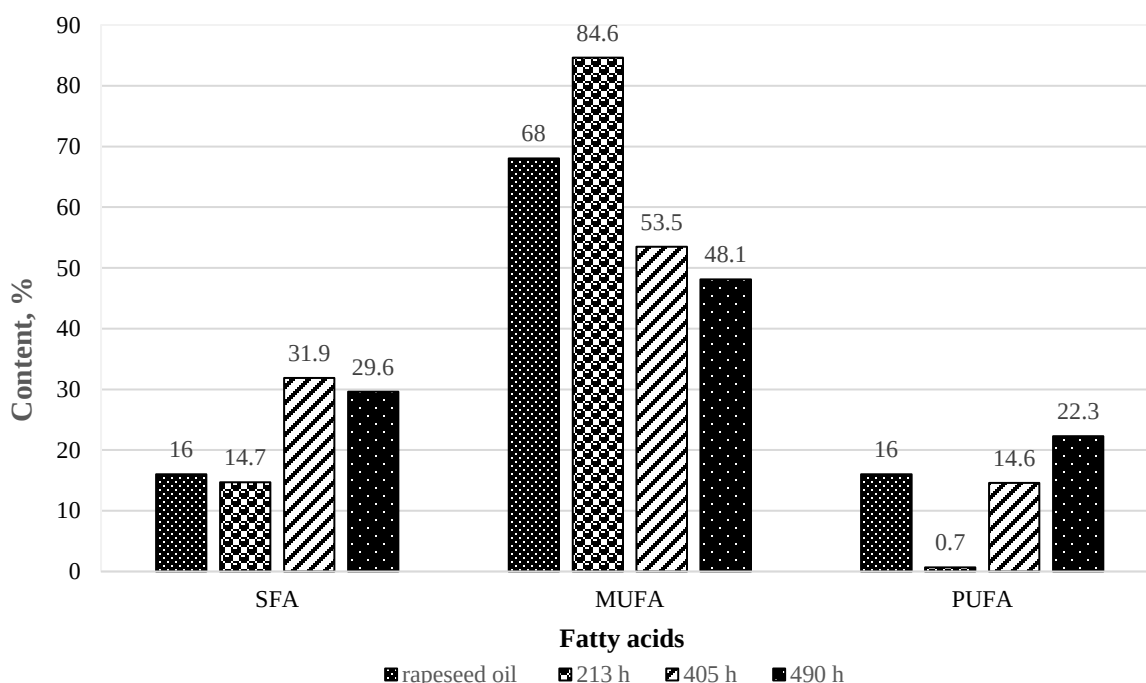


Figure 2. Saturated, mono- and polyunsaturated fatty acids in rapeseed oil and residual lipids isolated from three salinomycin samples during biosynthesis process (213 h, 405 h and 490 h).

* - SFA - Saturated fatty acids, MUFA - Monounsaturated fatty acids, PUFA - Polyunsaturated fatty acids

Table 3. Tocopherol and carotenoid contents and tocopherol composition of isolated lipids from the culture fluid during the biotechnological process

Samples	Tocopherols, mg.kg ⁻¹	Carotenoids, mg.100g ⁻¹
rapeseed oils	890±5 ^a	56.0±0.5 ^d
213 h	1038±20 ^b	46.6±0.3 ^c
405 h	3892±40 ^d	26.4±0.2 ^b
490 h	2379±10 ^c	22.8±0.1 ^a

Tocopherol composition, %				
	α-T*	β-T	γ-T	δ-T
rapeseed oil	33.5±0.5 ^a	-**	56.0±0.6 ^d	10.5±0.3 ^b
213 h	27.6±0.4 ^b	-	72.4±0.5 ^a	-
405h	19.5±0.5 ^c	3.9±0.2 ^a	61.6±0.5 ^c	15.0±0.4 ^a
490 h	18.4±0.3 ^d	2.1±0.1 ^b	64.3±0.2 ^b	15.2±0.3 ^a

* α-T - α-tocopherol, β-T - β-tocopherol, γ-T - γ-tocopherol, δ-T - δ-tocopherol

** - not detected

Values are means ± SD (*n* = 3). Different letters in the same column mean significant differences (*p* < 0.05) using Duncan test.

Total tocopherol and carotenoid contents of the residual lipids isolated from three salinomycin samples are given in Table 3.

The total amount of tocopherols during the fermentation process increased from 890 mg/kg (rapeseed oil) to 1038 mg.kg⁻¹ (213 h), 3892 mg.kg⁻¹ (405 h) and 2379 mg.kg⁻¹ (490 hours). The major component of tocopherols from rapeseed oil and in the isolated lipids from the culture fluid γ-tocopherol increased during the fermentation process from 56.0% (rapeseed oil) to 72.4% (213 h), then slightly decreased to 61.6% (405 h) and 64.3% (490 h), respectively, while the amount of α-tocopherol decreased from 33.5% (rapeseed oil) to 27.6% (213 h), 19.5% (405 h) and 18.4% (490 h) (Table 3).

Content of β-tocopherol and δ-tocopherol increased at the expense of α-tocopherol. The amount of γ-tocopherol decreased during the biosynthesis process but increased with comparison to that of the rapeseed oil.

The content of carotenoids decreased two and a half times compared to that of the initial rapeseed oil (56.0 mg/100g) and also decreased twice during

fermentation from 46.6 mg.100g⁻¹ to 22.8 mg.100g⁻¹ at the end of the process.

The content of phospholipids isolated from the different samples of the culture samples during the salinomycin fermentation process was 1.5%, 7.4% and 10.1%, respectively (Table 4). It is seen that the amount of phospholipids during the fermentation process increases. The data about the individual composition of the phospholipids (PL) isolated from the samples during the biotechnological process are presented in Table 4.

Table 4. Individual phospholipid composition of lipids isolated from the culture fluid at different stages of the fermentation process

PL, %	213 h	405 h	490 h
PC*	18.8±0.3 ^a	30.3±0.4 ^b	19.1±0.2 ^a
PE	81.2±0.5 ^a	27.7±0.2 ^c	47.7±0.5 ^b
PI	—**	10.5±0.2 ^a	5.5±0.1 ^b
PS	—	14.8±0.3 ^a	3.3±0.1 ^b
PA	—	7.6±0.1 ^a	2.3±0.1 ^b
LPC	—	4.8±0.1 ^b	7.0±0.2 ^a
LPE	—	4.3±0.1 ^b	15.1±0.2 ^a
Total PL	1.5±0.1	7.4±0.2	10.1±0.3

*-PC-phosphatidylcholine, PE-phosphatidylethanolamine, PI-phosphatidylinositol, PS-phosphatidylserine, PA-phosphatidic acids, LPC-lysophosphatidylcholine, LPE-lysophosphatidylethanolamine

** - not detected

Values are means ± SD (*n* = 3). Different letters in the same column mean significant differences (*p* < 0.05) using Duncan test

In the phospholipids isolated from the culture fluid at the 405 h and at the end of the fermentation process, 7 of the main classes of phospholipids were identified, while from the 213 h - only 2 phospholipids. In the phospholipid fraction from the 213 h and from the 490 h, phosphatidylethanolamine predominates, while in the fraction from 405 h, phosphatidylcholine (PC) and phosphatidylethanolamine (PE), are in commensurable amounts. During fermentation, the content of phosphatidylcholine increases from 18.8% (213 h) to 30.3% (405 h) and in the end of process it drops down to 19.1%, while phosphatidylethanolamine decreases from 81.2%,

(213 h) to 27.7% (405 h) and 47.7% (490 h). Most likely, some of these phospholipids such as phosphatidylcholine and phosphatidylethanolamine towards the end of the process, when the fermentation slows down, are used by the yeast as nutrients, therefore their amount decreases. A significant content of the lysoforms of phosphatidylcholine and phosphatidylethanolamine was also observed in the phospholipids isolated from the samples at 405 h (4.8 and 4.3%) and at the end of the process (7.0 and 15.1% respectively). Most likely, this is due to hydrolysis processes during the biosynthesis of salinomycin, since the nutrient medium contains a large amount of water, which leads to a high percentage of hydrolysis products.

An analysis of the fatty acid composition of the two main representatives of phospholipids (PC and PE) was also carried out in order to better understand the changes that occur in them during the fermentation process. Table 5 describes in detail the changes that occur in the fatty acid composition of the two main phospholipids.

The main phospholipid classes of the studied samples contain mainly palmitic (C_{16:0} – 28.3 – 64.4%), stearic (C_{18:0} – 2.7 – 14.0%) and oleic (C_{18:1} – 8.0 – 51.7%) acids. The content of palmitic acid in the phosphatidylcholine (from 33.6% (213 h) to 64.4% (490 h)) and the phosphatidylethanolamine [from 28.3% (213 h) to 40.0% (490 h)] increases in the process of antibiotic biosynthesis at the expense of oleic acid which decreases significantly (from 47.6% (213 h) to 8.0% (490 h) in phosphatidylcholine and from 51.7% (213 h) to 40.3% (490 h) in phosphatidylethanolamine). The amount of palmitic acid in phosphatidylcholine is higher than that in phosphatidylethanolamine during the entire fermentation process. The content of stearic acid in the phosphatidylcholine decreases [from 7.3% (213 h) to 2.7% (490 h)], while in the phosphatidylethanolamine initially increases [from 10.5% (213 h) to 14.0% (405 h)] then decreases to 3.7% (490 h). The amount of linoleic acid in the phosphatidylcholine initially increases [from 0.3% (213 h) to 2.7% (405 h)] then decreases to 0% (490 h), while in the phosphatidylethanolamine increases from 0% (213 h) to 3.6% (490 h). The amount of linolenic acid in the two phospholipid classes decreases from 4.6% (213 h) to 0.2% (490 h) and from 3.5% (213 h) to 1.9% (490 h) respectively.

Table 5. Fatty acid composition of the main phospholipids classes isolated from culture fluid samples

Samples Fatty acids**, % wt	213 h		405 h		490 h	
	PC*	PE	PC	PE	PC	PE
C _{8:0}	***	0.1±0.1	-	0.2±0.1	-	-
C _{10:0}	0.1±0.1	-	-	5.1±0.1	0.5±0.1	-
C _{12:0}	-	-	-	-	0.7±0.1	-
C _{14:0}	0.1±0.1	0.5±0.1	0.5±0.1	0.5±0.1	2.9±0.1	5.5±0.1
C _{14:1}	1.9±0.1	1.6±0.1	3.5±0.1	1.1±0.1	5.5±0.1	1.5±0.1
C _{15:0}	0.3±0.1	0.2±0.1	0.5±0.1	0.4±0.1	0.6±0.1	0.4±0.1
C _{15:1}	0.2±0.1	0.2±0.1	0.4±0.1	0.2±0.1	9.4±0.2	0.3±0.1
C _{16:0}	33.6±0.4	28.3±0.4	59.2±0.5	29.4±0.4	64.4±0.5	40.0±0.4
C _{16:1}	-	0.5±0.1	-	0.4±0.1	-	0.2±0.1
C _{17:0}	1.2±0.1	0.7±0.1	1.0±0.1	0.5±0.1	0.7±0.1	0.5±0.1
C _{17:1}	0.1±0.1	0.2±0.1	2.3±0.1	0.1±0.1	3.6±0.1	0.2±0.1
C _{18:0}	7.3±0.2	10.5±0.2	3.9±0.1	14.0±0.2	2.7±0.1	3.7±0.1
C _{18:1}	47.6±0.5	51.7±0.5	25.0±0.3	41.0±0.3	8.0±0.1	40.3±0.5
C _{18:2}	0.3±0.1	-	2.7±0.1	0.2±0.1	-	3.6±0.1
C _{18:3}	4.6±0.1	3.5±0.1	0.2±0.1	3.2±0.1	0.2±0.1	1.9±0.1
C _{20:0}	-	-	-	-	-	0.1±0.1
C _{20:2}	-	-	-	0.3±0.1	-	0.2±0.1
C _{22:0}	2.7±0.2	2.0±0.3	0.8±0.1	3.4±0.2	0.8±0.1	1.6±0.2

* - PC - phosphatidylcholine, PE - phosphatidylethanolamine

** - C_{8:0}—caprylic; C_{10:0}—capric; C_{12:0}—lauric; C_{14:0}—myristic; C_{14:1}—mirystioleic; C_{15:0}—pentadecanoic; C_{15:1}—pentadecenoic; C_{16:0}—palmitic; C_{16:1}—palmitoleic; C_{17:0}—margaric; C_{17:1}—heptadecenoic; C_{18:0}—stearic; C_{18:1}—oleic; C_{18:2}—linoleic; C_{18:3}—linolenic; C_{20:0}—arachidic; C_{20:2}—eicosadienoic; C_{22:0}—behenic.

*** - not detected

The amount of myristic acid in the PC and the PE increases from 0.1% to 2.9% and from 0.5% to 5.5% respectively. The content of the remaining saturated and unsaturated fatty acids in the phospholipid fraction are represented in insignificant amounts.

The ratio between saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) of the main two phospholipids isolated from the culture fluid at different stages of the fermentation process is presented in Fig. 3.

During the fermentation process, the amount of saturated fatty acids in both major phospholipid classes increases mainly at the expense of a decrease in the amount of monounsaturated fatty acids. The amount of saturated fatty acids in the phosphatidylcholine, isolated from sample in 213

hour was 45.3% and reached 73.3% at the end of the process, and the amount of unsaturated fatty acids decreased from 54.7% (213 h) to 26.7% (490 h). The quantity of saturated fatty acids in the phosphatidylethanolamine, isolated from sample in 213 hour was 42.3% and reached 53.5% at 405 h then slightly decreased to 51.7% at the end of the process. The amount of unsaturated fatty acids in the same phospholipid decreased from 57.7% (213 h) to 46.5% (405 h) and at the end of process slightly increased to 48.3% (490 h). The amount of monounsaturated and polyunsaturated fatty acids in PC decreased (from 49.8% to 26.5% and from 4.9% to 0.2%, respectively), while in PE the amount of monounsaturated fatty acids decreased from 54.2% to 42.5%, and that of polyunsaturated fatty acids increased from 3.5% to 5.8%.

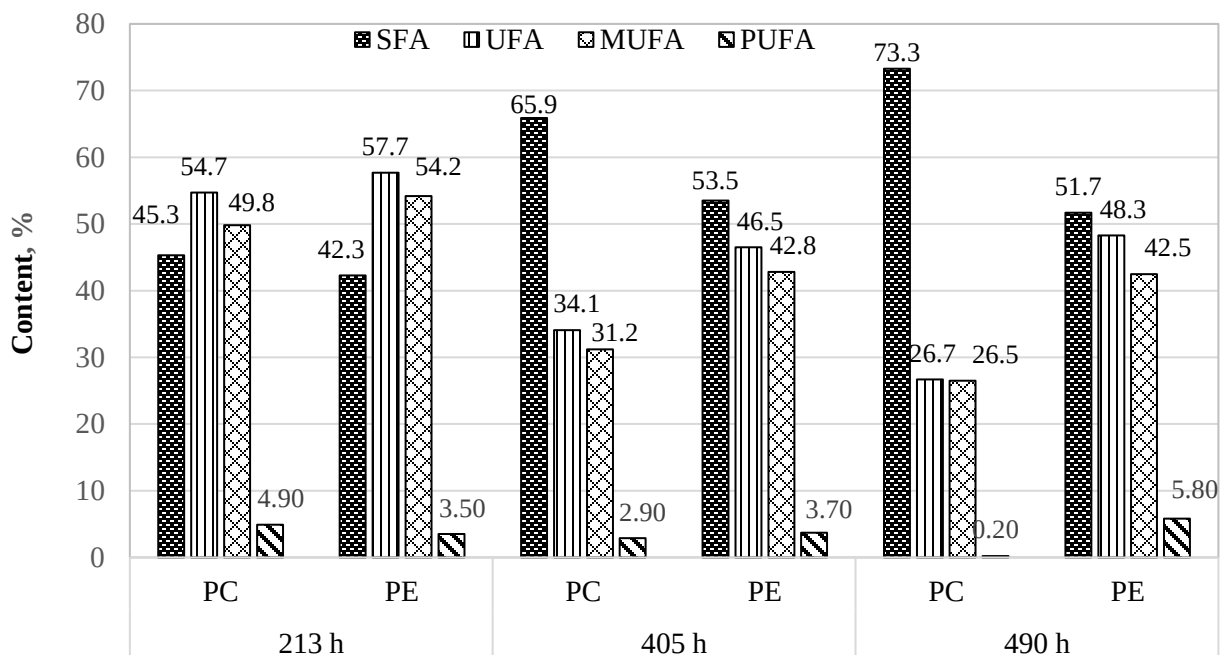


Figure 3. Ratio between saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) of the main two phospholipids (PC and PE) isolated from the culture fluid at different stages of the fermentation process

Conclusions

Significant changes in the general lipid composition were observed during the biosynthesis process of salinomycin. The content of triacylglycerols was significantly reduced during fermentation – from 96.0% to 2.3%. The main trend in fatty acid composition was an increase in the amount of saturated fatty acids [14.7% at 213 h, 31.9% at 405 h and 29.6% at the end of the process (490 h)] at the expense of a decrease in monounsaturated acids (respectively 84.6%, 53.5% and 48.1%). The most significant changes were in the amounts of oleic acid (from 67.5% to 46.2%) and stearic acid (from 3.7% to 18.4%). The content of saturated (palmitic and stearic) and polyunsaturated (linoleic) fatty acids increased while monounsaturated (oleic) fatty acids decreased. The content of tocopherols increased during fermentation process while the content of carotenoids decreased. The content of phospholipids in the process of obtaining the antibiotic salinomycin increases as well. The phosphatidylcholine and phosphatidylethanolamine were identified as major polar lipid components. The main phospholipid classes contain mainly

palmitic, stearic and oleic acid. The saturated fatty acids predominate in both phospholipid components (PC and PE) at the end of the process. A higher content of saturated fatty acids is recommended in the biosynthesis process of salinomycin, as this provides higher fluidity and quality of the final product.

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