Bulgarian Chemical Communications, Volume 51, Special Issue D (pp. 273 – 276) 2019

Composition of residual lipids isolated from *salinomycin*

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Received November 15, 2018; Revised January 4, 2019

Salinomycin is an antimicrobial polyether ionophore antibiotic that is synthesized by microbiological pathway, and vegetable oils (mainly rapeseed oil) are used as a nutrient medium. Some of the lipids remain in the final product and affect its quality (fluidity). The composition of residual lipids isolated from three *salinomycin* samples of different fluidity (with good fluidity (80%), with bad fluidity (<80%) and imported sample with good fluidity (>80%)) was studied. The general lipid composition of rapeseed oil used for the nutrient medium in the preparation of *salinomycin* is: triglycerides - 96.0%, unsaponifiables - 1.7%, free fatty acids - 0.5%, phospholipids and other polar compounds - 1.2%. The lipid content of the *salinomycin* samples is 30.2%, 40.5% and 21.6% respectively and they have the following composition: monoglycerides - from 5.1 to 12.7%, diglycerides - from 8.3 to 14.8%, triglycerides - from 10.4 to 25.2%, free fatty acids - from 1.4 to 14.7%, polar compounds - from 45.9 to 62.2%. Significant changes in fatty acid composition were observed during the biosynthesis process. The amount of unsaturated fatty acids (oleic and linoleic) in the oils isolated from the samples with good viscosity decreased significantly at the expense of the increased content of saturated fatty acids - stearic, palmitic and especially capric acid (from 0.1% to 2.9 - 14.1%). It can be concluded that in the process of *salinomycin* biosynthesis, the lipids undergo significant transformations - triacylglycerols are hydrolyzed and the long-chain unsaturated fatty acids are degraded to medium-chain saturated fatty acids.

Keywords: salinomycin, lipids, fatty acid composition

INTRODUCTION

The antibiotic *salinomycin* is an antibacterial and coccidiostat ionophore therapeutic drug. *Salinomycin* exhibits high antimicrobial activity against Gram-positive bacteria and is used in birds and other animals. The "Biovet" Peshtera produces two products based on *salinomycin* with the market brand Sacox - Sacox®120 microgranulate and Sacox®200 microgranulate. They are anticoccidial feed additives for the prophylactic control of coccidiosis in chickens for fattening and chickens reared for laying. Sacox 120 and Sacox 200 microgranulate contain 12% and 20% of *salinomycin*, sodium and calcium carbonate and silicon dioxide as carriers. These components are produced by means of microgranulation, which are inseparably combined and this lead to improving the activity of the product. This production process results in a uniform microgranulate with excellent flow characteristics.

The production of *salinomycin* was carried out by fermentation in a known manner [1-3], wherein vegetable oils such as soybean oil, sesame oil, rapeseed oil, safflower oil, olive oil, methyl oleate, methyl myristate and methyl linoleate are used as carbon sources and as defoaming agents. The formed agglomerates with polyether antibiotics include glyceride fats, free fatty acids, and phospholipids such as lecithin. *Salinomycin* from "Biovet" Peshtera is produced by using mainly rapeseed and soybean oil as culture medium. The final product contains a residual amount of lipids that affect its fluidity, so it is necessary to determine their composition.

The purpose of this work is to determine the composition of the residual lipids isolated from *salinomycin* with different fluidity.

EXPERIMENTAL

Samples

Three *salinomycin* samples with different fluidity (with good fluidity (80%), with bad fluidity (<80%) and imported sample with very good fluidity (>80%)) were studied. The first two were manufactured by "Biovet" Peshtera and the third was imported from China.

Methods

Isolation and quantification of lipid classes by thin-layer chromatography (TLC)

The residual lipids were extracted with hexane in a *Soxhlet* apparatus for 8 h [4]. After that the solvent was removed by a rotary evaporator and the residue was weighed to determine the oil content of each sample. The main lipid classes were identified by applying 1 mL of the residual lipids solution in hexane on a 20 cm × 20 cm glass plate with ca. 1 mm thick silica gel 60 G layer and developed with hexane-acetone, 100:8 (v/v). The mixture of lipid

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classes containing equal parts of docosane-, cholesterol oleate-, triolein-, cholesterol-, 1,3-diolein-, 1-monooleinrac-glycerol and oleic acid were used as references. Triacylglycerols, sterols, diacylglycerols, monoacylglycerols, free fatty acids and polar lipids (eluted in this order) were unambiguously identified and isolated by elution with diethyl ether. The solvent was evaporated under a stream of nitrogen and the residues were weighed in small glass containers to constant weight [5].

Gas chromatography of fatty acid methyl esters

The fatty acid composition of lipids was determined by gas chromatography (GC) after transmethylation of the respective sample with 2% H_2SO_4 in CH_3OH at 50ºC [6]. Fatty acid methyl esters (FAME) were purified by TLC. GC was performed on an HP 5890 series II gas chromatograph unit equipped with a 60 m × 0.25 mm (I.D.) × 25 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 to 240°C (10 min); injector and detector temperatures are kept at 250°C. Identification of fatty acids was performed by comparison of retention times with those of a standard mixture of fatty acids subjected to GC under identical experimental conditions [7]. The analytical standard of fatty acid methyl esters was purchased from Sigma-Aldrich Chemical Co. All solvents and reagents were of analytical grade from Merck and were used without additional purification.

Determination of salinomycin by HPLC

Antibiotic content in the products was determined by high performance liquid chromatography. Chromatographic system is Shimadzu, equipped with a chromatographic column-Chromolith RP-18 100×4.6 mm. Mobile phase contained a mixture of methanol, water and acetic acid – 90:10:0.1 (v/v/v). Chromatographic conditions were: flow of the mobile phase 0.850 mL/min, flow of the derivatizing solution 0.900 mL/min, analysis temperature 98°C and wavelength 520 nm [8].

RESULTS AND DISCUSSION

General characteristics of the oil and residual lipids

The content of antibiotic and residual lipids, isolated from Sacox with different fluidity is shown in Table 1. The sample with bad fluidity has the greatest oil content while the sample with very good fluidity has the smallest quantity of the oil. It was found that the antibiotic *salinomycin* was present in an amount from 11.9 to 18.8% in the tested samples. The results about antibiotic content in the examined samples are in agreement with the quality certificate for *salinomycin* content in the marked products. The level of antibiotic content is lower than that of samples with very good fluidity. The general lipid composition of rapeseed oil used for the nutrient medium in the preparation of *salinomycin* is: triglycerides - 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%).

Table 1. Content of antibiotic and residual lipids, isolated from Sacox with different fluidity

Samples	Residual lipids from Sacox, %	Antibiotic content, %
Good fluidity (80%)	30.2	18.8
Bad fluidity (<80%)	40.5	18.6
Very good fluidity (>80%)	21.6	11.9

The general lipid composition of residual lipids isolated from *salinomycin* with different fluidity is shown in Table 2. The significant changes in lipid composition between the initial rapeseed oil and the isolated lipids from the tested samples have been observed during the biosynthesis process. The content of triacylglycerols significantly reduces during the process while this of mono-, diglycerides and free fatty acids increases. The residual lipids from samples of the *salinomycin* with good and very good fluidity have the higher quantities of polar compounds and free fatty acids.

Table 2. Lipid composition of residual lipids isolated from *salinomycin* with different fluidity

Component, %	Product Sacox		
	Good fluidity	Bad fluidity	Very good fluidity
Monoglycerides	5.1	12.7	6.6
Diglycerides	8.3	14.8	8.7
Triacylglycerols	10.4	25.2	14.9
Free fatty acids	14.0	1.4	14.7
Polar compounds	62.2	45.9	55.1

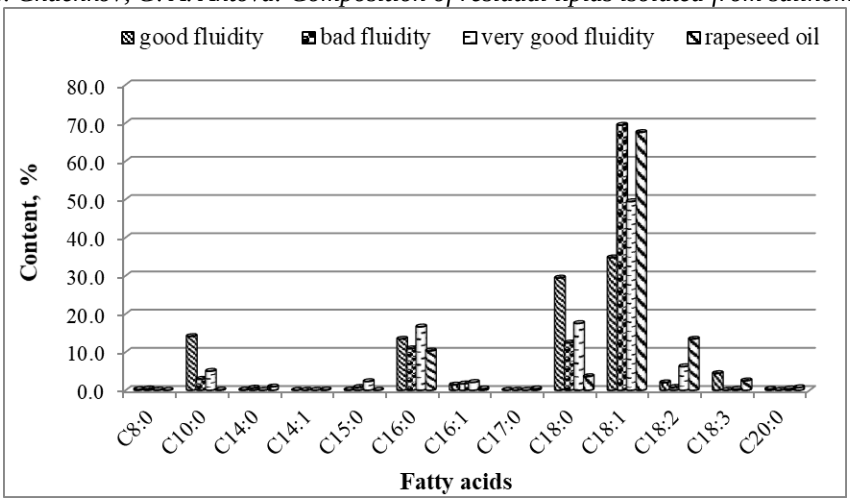

Figure 1. Fatty acids composition of rapeseed oil and residual lipids isolated from *salinomycin* with different fluidity: *$C_{8:0}$ – caprylic, $C_{10:0}$ – capric, $C_{14:0}$ – myristic, $C_{14:1}$ – myristoleic, $C_{15:0}$ – pentadecanoic, $C_{16:0}$ – palmitic, $C_{16:1}$-palmitoleic, $C_{17:0}$ – margaric, $C_{18:0}$ – stearic, $C_{18:1}$ – oleic, $C_{18:2}$ – linoleic, $C_{18:3}$ – linolenic, $C_{20:0}$ – arachidic acid

The changes in the fatty acid composition of rapeseed oil and residual lipids isolated from salinomycin with different fluidity have also been investigated (Figure 1). Oleic acid (67.5%) and linoleic acid (13.4%), followed by palmitic acid (10.3%) and stearic acid (3.6%), were found to predominate in the fatty acid composition of the rapeseed oil.

Oleic acid is in the highest amount in all samples (in the sample with good fluidity it is 34.7%, with very good fluidity - 49.4% and with bad fluidity - 69.4%, respectively). The residual lipids from the different samples contain palmitic acid between 10.9% (with bad fluidity) and 16.6% (with very good fluidity) and a significantly high amount of stearic acid - between 12.4% (with bad fluidity) and 29.4% (with good fluidity). The amount of unsaturated fatty acids (oleic and linoleic) in the oils isolated from the samples with good viscosity decreased significantly at the expense of increasing of the content of saturated fatty acids - stearic, palmitic and especially capric acid (from 0.1% (rapeseed oil) to 2.9 - 14.1% (with good fluidity)).

Figure 2 shows the ratio of unsaturated and saturated fatty acids in rapeseed oil and residual lipids isolated from *salinomycin.* In the composition of rapeseed oil used as a nutrient medium, unsaturated fatty acids predominate (83.9%). In the process of synthesizing *salinomycin*, their amount decreases significantly. In the residual lipids isolated from *salinomycin* with good and very good fluidity the amount of unsaturated fatty acids is 42.5% (good fluidity) and 58.0% (very good fluidity), respectively. The quantity of saturated fatty acids increases from 16.1% to 42.0% and 57.5% (with good fluidity). The ratio of saturated: unsaturated fatty acids in rapeseed oil and isolated lipids from the sample with poor fluidity is 16.1:83.9, i. e. 1:5.2 and 28.0:72.0, i. e. 1:2.6, respectively, while in the lipids of the sample with very good fluidity it is much lower 42.0:58.0, i. e. 1:1.4. Lower content of unsaturated (42.5%) and respectively higher content of saturated fatty acids (57.5%) is found in the lipids isolated from the sample with good fluidity (1:0.7).

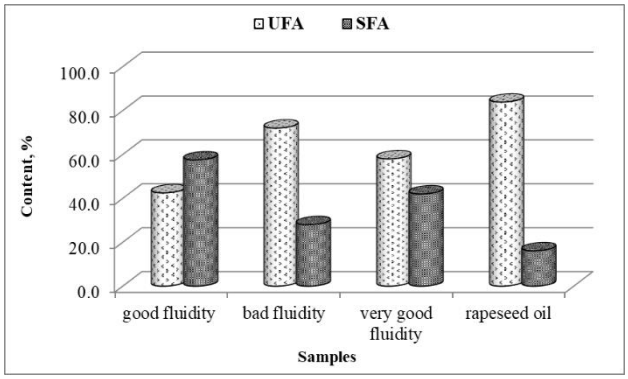

Figure 2. Ratio between unsaturated (UFA) and saturated (SFA) fatty acids in rapeseed oil and in residual lipids isolated from *salinomycin* with different fluidity

CONCLUSIONS

The lipids undergo significant transformations in the process of *salinomycin* biosynthesis - triacylglycerols are hydrolysed and the long-chain unsaturated fatty acids are degraded to medium-chain saturated fatty acids.

In conclusion, the use of vegetable oils with a higher content of saturated fatty acids (palmitic and stearic fatty acid) is recommended in the process of *salinomycin* biosynthesis, which ensures higher fluidity and quality of the final product.

Acknowledgement: *We are grateful to "Biovet" Peshtera for the provided samples for carrying out this study.*

REFERENCES

1. Y. Miyazaki, M. Shibuya, H. Sugawara, O. Kawaguchi, Ch. Hirose, J. Nagatsu, S. Esumi, *Journal of Antibiotics* (Tokyo), **27**, 814 (1974).
2. H. Kinashi, N. Otake, H. Yonehara, *Tetrahedron Letters*, **49**, 4955 (1973).
3. H. Kinashi, N. Otake, *Agr. Biol. Chem*. **40**, 1625 (1976).
4. Company standard "Biovet" Peshtera QCD-FPC-0878-09. Determination of crude fat content.
5. M. Kates, Techniques of lipidology: Isolation, analysis and identification of lipids, North-Holland Pub. Co., American Elsevier Publishing Co., Inc.-New York, 1972.
6. ISO 12966-2:2011. Animal and vegetable fats and oils. Gas chromatography of fatty acid methyl esters Part 2: Preparation of methyl esters of fatty acids.
7. ISO 12966-1:2014. Animal and vegetable fats and oils. Gas chromatography of fatty acid methyl esters. Part 1: Guidelines on modern gas chromatography of fatty acid methyl esters.
8. Company standard "Biovet" Ltd. Peshtera QCD-FPC-0878-03. Determination of the contents and impurities of salinomycin by HPLC.