LIPID FRACTIONS ANALYSIS IN PORK MEAT BY HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY

ANALIZA FRACŢIUNILOR LIPIDICE DIN CARNEA DE PORC PRIN CROMATOGRAFIE ÎN STRAT SUBŢIRE DE INALTĂ PERFORMANŢĂ

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Abstract. *High performance thin layer chromatography is a fast and eficient method for the separation of complex mixtures. The HPTLC analysis of lipid fractions on silica gel plates used for extraction a solvent mixture consisting of hexane, chloroform and ethanol and as reagent color a mix of copper sulphate and phosphoric acid. The resulting chromatogram presented a variable baseline and five corresponding peaks to the following lipid fractions: cholesterol esters- triacylglicerols, free fatty acids, cholesterol, phospholipids, mono- and diacylglicerols. Interpretation of the HPTLC analysis results can provide information about both the (%) composition of the major lipid fractions and about the freshness of the samples analyzed (meat of Landrace pork raised with industrial diet or in tradional farm versus meat of Vietnamese pork raised in tradional farm).*
Key words: *meat , pork, lipids, chromatography, HPTLC.*

Rezumat. *Cromatografia în strat subţire de înaltă performanţă (HPTLC) este o metodă rapidă şi eficientă de separare a amestecurilor complexe. Analiza realizată pe plăci HPTLC silicagel 60, cu folosirea amestecului de solvenţi de developare, format din hexan-cloroform-etanol şi a reactivului de culoare reprezentat de sulfat de cupru-acid fosforic a condus la obţinerea de cromatograme cu linia de bază variabilă şi cinci picuri cromatografice corespunzătoare urmatoarelor fracţiuni lipidice: triacilgliceroli, esterii colesterolului, acizi graşi liberi, colesterol, mono- şi diacilgliceroli, fosfolipide. Interpretarea rezultatelor analizei HPTLC poate oferi informaţii atât despre compoziţia procentuală a principalelor fracţiuni lipidice cât şi despre starea de prospeţime a probelor analizate (carne de porc Landrace crescut cu dietă industrializată / fermă tradţională vs carne de porc Vietnamez crescut în gospodărie individuală).*
Cuvinte cheie: *carne, porc, lipide, cromatografie, HTPLC.*

INTRODUCTION

Determination of food substrates composition is a priority of ensuring food safety and quality. Meat chemical composition can vary depending with the animal specie, breed, age, sex, food regime or just with the anatomical portion

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taken into consideration. In meat, the unique association of several types of (muscle, fat, bone, loose or connective) tissues provides a solid nutritional base, mainly because of the richness in protein and low in carbohydrate content.

Investigation of the meat's physic + chemical properties can provide information both on the chemical composition but also on its freshness. The new analytical methods ensure fast accurate measurements with low reagent consumption as compared to standard classical techniques. High performance thin layer liquid chromatography (HTPLC) is a method certified for many industrial biotechnological processes (Byrdwell, 2005) used for the separation of the complex mixtures.

The aim of this study was to determine the main lipid fractions of the pork meat (Landrace pigs raised with industrial diet, Landrace pigs raised in tradional farm and Vietnamese pigs raised in rational farm) by HPTLC a fast, effective (Damyanova, 2003) and accurate (Deinstrop, 2007) method that offers information on the composition of the lipid fractions and on the freshness degree of the analyzed samples (Fuchs et al., 2011).

MATERIAL AND METHOD

Material for analysis consisted of meat samples extracted from the same haunch area (neck muscles) of 10 adult male pigs (Landrace and Vietnamese pig) that were raised with industrial diet in individual household or in tradional farms system.

Determination of the chemical composition by HPTLC

For lipids extraction, in the developing tank on the silica gel plates, a series of solvents were used: hexane, cloform and ethanol while for developing and fixing stage, the solutions used were copper sulphate, phosphoric acid and ethanol.

By HPTLC method the main lipids fraction determined were tri-, di- and mono-acylglycerols, cholesterol, free fatty acids and phospholipids.

Procedure (Hillenkamp and Katalini , 2007; Leo and Nolet, 2007):

• sample preparation for analysis (extraction): in a microtube (Fig. 2) 0.5 diluted sample with distilled water and 1 ml solvent was inserted. Samples were weighed to determine the dilution performed (sample mass + mass of water added). The samples were subjected to centrifugation for four minutes at 4000 rotation per minut;

• preparation of chromatographic plate: 0.25 ml was taken from the clear top of the centrifuged sample. This quantity was spott using a micropipette on the plate (two points for each sample). The board was divided into a number of regions equal to the number of samples;

• plate developing: the plate with spotlights was inserted with a clip and a loop into the developing tank (Fig. 1), that contaned the developing solvent (60 ml hexane, 36 ml of chloroform and 4 ml ethanol).

After solvent migration, the chromatographic plate was removed from the tank and was washed by spraying with the developing solution (10 g $CuSO_4$ is mixed with the H_3PO_4 solution in a 100 ml graduated flask).

After spraying, the plate was coated with a filter paper and kept in the oven at 120°C for five minutes in order to assure the best analysis of the major lipid fraction's characteristic bands and spots.

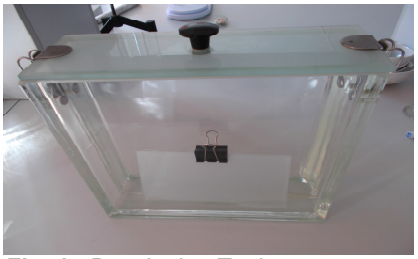

Fig. 1 - Developing Tank

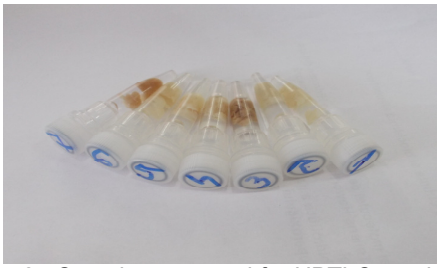

Fig. 2 - Samples prepared for HPTLC analysis

RESULTS AND DISCUSSIONS

Figure 3 presents the image of chromatographic plate after drying stage, with the characteristic lipid fractions' major bands and spots.

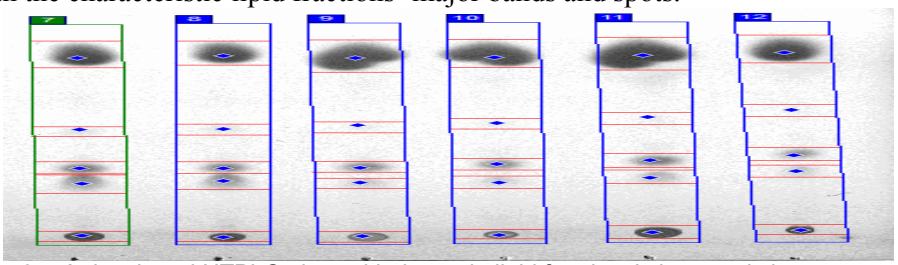

Fig. 3 - A developed HTPLC plate with the main lipid fractions' characteristic bands and spots

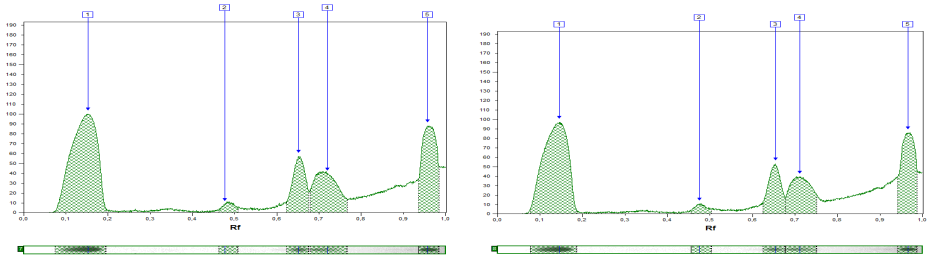

Fig. 4 - The chromatograms of the main lipid fractions for the bands number 7 and 8 (Vietnamese pork meat)

For Vietnamese pork meat, chromatograms (Fig. 4) of the two bands were very similar (same sample of meat). They consist of variable base-line and five chromatographic peaks (corresponding to the number of lipid fractions: TAG + CE, FFA, COL, MAG + DAG and PL). Only the peaks number three and four presented inseparable baseline, the other peaks showed well defined peaks.

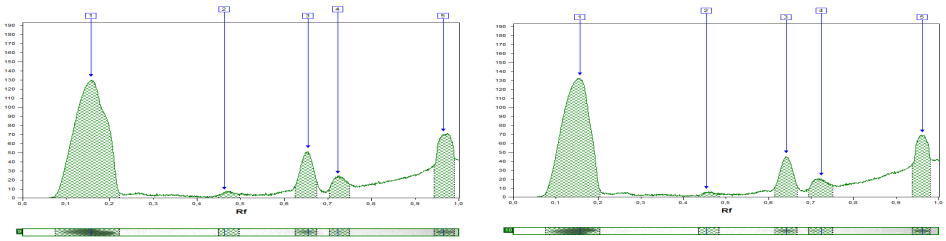

Fig. 5 - The chromatograms of the main lipid fractions for the bands number 9 and 10 (industrial pig diet meat)

As it shown in Figure 5 (industrial pig diet meat) the chromatograms of the two strips are almost identical with little differences. They also include five chromatographic peaks and a variable baseline. All baseline peaks were perfectly represented.

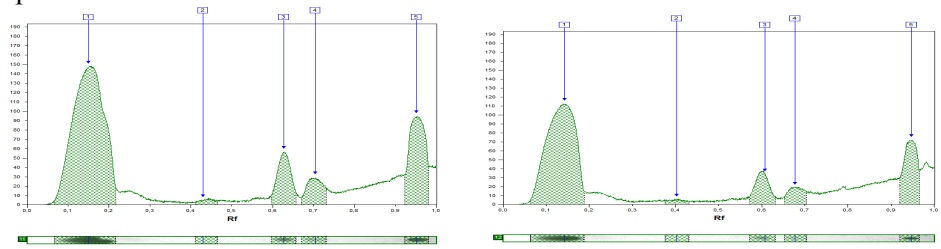

Fig. 6 - The chromatograms of the main lipid fractions for the bands number 11 and 12 (traditional farm raised pork's meat)

Also for the traditional farm pork's meat the chromatograms of both bands were similar, with a variable baseline and with five peaks corresponding to the main lipidic fractions (Fig. 6). The area of the peak number one (TAG + CE) had the highest proportion, followed by number five peak's surface (PL). The corresponding surface of the peak number two (FFA) had the lowest proportion.

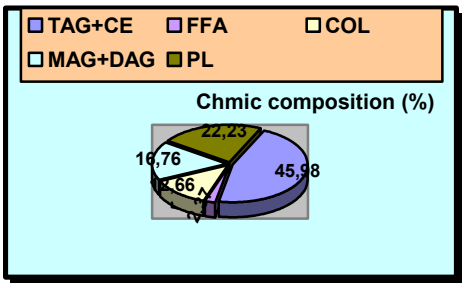

Fig. 7 - The main lipid fractions proportion (%) for Vietnamese pig's meat

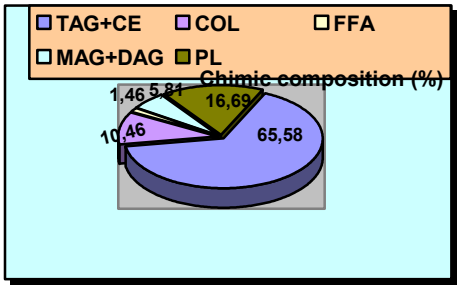

Fig. 8 - The main lipid fractions proportion (%) for industrial diet pig's meat

Analysing the lipid fractons' proportion for Vietnamese pork (fig. 7), the highest value was recorded for triacylglycerols (45.98 %), followed by

phospholipid (22.23 %), mono + diacylglycerols (16.76 %) and cholesterol (12.66%). Lowest proportion was obtained for free fatty acids (2.37 %).

In the case of the industrial diet pig's meat (fig. 8), the highest value recorded for lipid fractions was for triacylglycerols (65.58%), a value that exceeds much more the half (50 %) of the total weight of the lipid fractions. The smallest weight proportion (1.46 %) was represented by the free fatty acids, followed by the mono and diacylglycerol (5.81%), the cholesterol (10.46 %) and the phospholipid (16.69 %). Figure 9 presents proportions (%) of the of the main lipid fractions for traditional farm pig's meat.

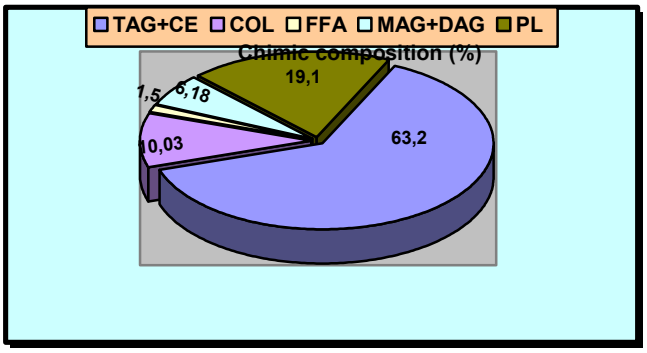

Fig. 9 - The main lipid fraction proportion of the traditional farm pig's meat

As for the proportion of lipid fractions of the traditional pork's meat (Fig. 9), the highest value registered was for triacylglycerols (63.2 %), followed by phospholipids (19.1 %), cholesterol (10.03 %) and mono+ diacylglycerols (6.18 %). Smallest proportion was for the free fatty acids (1.5 %). Figure 10 shows the values of cholesterol and tri+di+ monoacylglycerols:

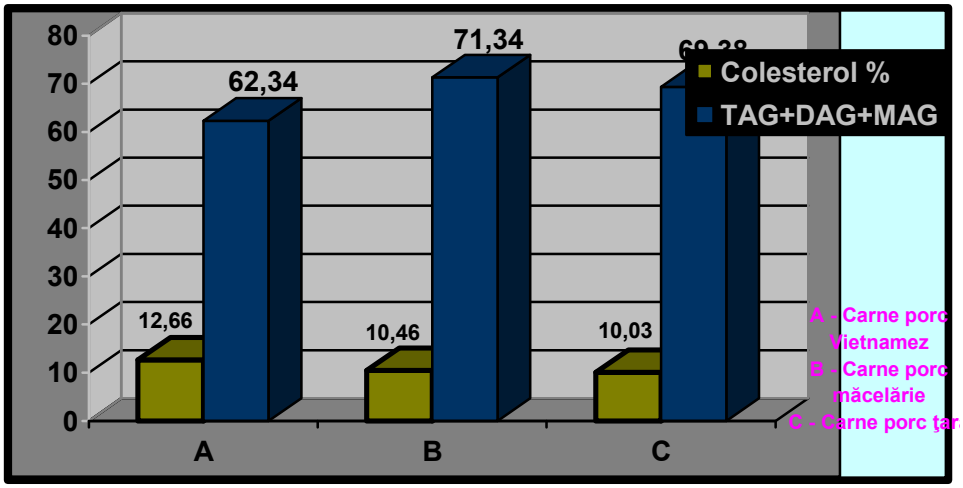

Fig. 10 - The Cholesterol and TAG+DAG+MAG content of the main types of meat's sample analyzed

The analysis results showed that the mean value of the cholesterol (fig. 10) in the pork meat was 11.05%. In the case of the traditional pork's meat the percentage of the cholesterol was the lowest (10.03 %), value very closed to the one's of industial diet pig meat (10.46 %). Our results (12.66 % cholesterol) are in contradiction with the various statements and recommendations that claimes the "almost free of cholesterol content" of the Vietnamese pork's meat. The proportion of TAG + DAG + MAG for Vietnamese pork' meat was 62.34 % compared to industrial diet pig meat (71.34 %) and traditional farm raised pork meat (69.38 %), which shows that lipogenesis and TAG deposit is to a lesser extent (Trincă and Ariton, 2014) in this species.

CONCLUSIONS

The analysis of the lipid fractions by HPTLC method showed that:

1. the mean value of the cholesterol content in the pork meat was 11.05%, with the highest value (12.66 %) for the Vietnamese pork meat and the smallest value for the farm traditional pork's meat .

2. the proportion of TAG + DAG + MAG for Vietnamese pork' meat was 62.34 % compared to industrial diet pig meat (71.34 %) and traditional farm raised pork meat (69.38 %), results that highlights the lesser extent of lipogenesis and TAG deposit in Vietnamese pork meat.

3. the content of mono+ di-acylglycerols and free fatty acids resulted from triacylglycerols had normal values characteristic to the freshness state for both the meat samples of the traditional farm pork and industrial diet pig. In the case of the the Vietnamese pork's meat products resulted from hydrolysis (di+mono-acylglycerols and free fatty acids) the registered values were about 3 times higher compared to the other types of meat, values correlated with the longer duration of storage (about the 3 times higher).

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