HIGHLIGHT OF BUFFALO MILK CHOLESTEROL THROUGH THIN-LAYER CHROMATOGRAPHY

Aurelia Pece, Vioara Mireşan, Camelia Răducu, C. Coroian, D. Cocan, R. Constantinescu

University of Agricultural Sciences and Veterinary Medicine, Faculty of Animal Science and Biotechnologies Cluj-Napoca
e-mail: aureliapece@yahoo.com

Abstract

The highlight of buffalo milk cholesterol was conducted by means of thin-layer chromatography and the migration of mobile phases along an absorbent layer. Free and esterised cholesterol extraction was conducted according to the method of Folch et. al., performing small alterations regarding solvent volume or the volume of samples under analysis. Many reactives underwent testing, as the initial iodine visualising was not appropriate. The quantitative identification was conducted by comparing the Rfs of free and esterised cholesterol from analysed samples with existing standards. Spots were highlighted using a photodensimeter.

Key words: cholesterol, milk, buffalo, CSS

MATERIALS AND METHODS

2.5 ml of milk were extracted with 48 ml solution chloroform/methanol/water (2:1:1, v/v/v) through intermittent stirring for 30 minutes in a separation funnel. The superior layer (methanol and water) was separated through siphonage, while the middle and inferior layers (proteins and chloroform containing free and esterised cholesterol) was vacuum-filtered through a Büchner funnel. The chloroform layer was filtered again through anhydrous sodium sulfate, while Na_2SO_4 is washed 3 times with 5 ml of chloroform each. Fatty extracts combined vacuum-evaporated using a rotaevaporator at 50ºC, while the residue was recovered with 1 ml chloroform, in a 2 ml vial with a raylon lid and kept at 18ºC before analysis. Over the residue obtained, 1 ml of metoxide methanol solution (0.5M) will be added alongside 1 ml methanol and 1 ml toluene and heated at 70ºC for an hour. After cooling, we add 1 ml distilled water and cholesterol is extracted with 2x8 ethyl ether in a separation funnel. Ether cholesterol extracts are filtered on anhydrous sodium sulfate and dry-evaporated.

The cholesterol can be removed from diet products by mixing homogenized milk with β-cyclodextrine, a procedure that removes 93-93% of the cholesterol (Jensen R.G., 2002; Lee D.K. şi col. 1999; Sieber R., 1994).

RESULTS AND DISCUSSIONS

Visualising fats was made through iodine vapour exposure in a chromatographic chamber covered with a glass lid. Free and esterised cholesterol, as well as triglycerides appear as brown spots (iodine complexes with the double links of the compounds under analysis).

Figure 1.1 Sepration through thin-layer chromatography of neuter fats in buffalo milk after sodium metoxide separation

The Rfs for neuter lipid standards are obtained through developing by means of a mixture of petroleum ether (pf 40-60ºC), ethyl ether and acetic acid in different proportions.

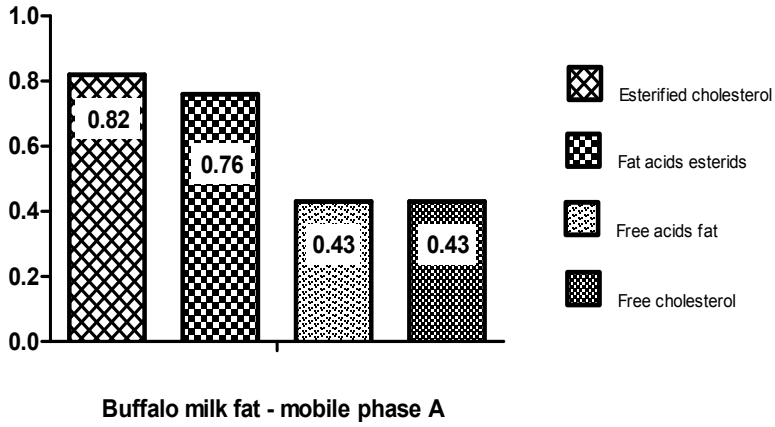

Figure 1.2 Variability of buffalo milk fats through the employment of mobile phase A

Several mobile phases were analysed in order to obtain a satisfactory separation, especially for free cholesterol and free fatty acids. This issue emerged as transesterification with sodium metoxide was used, which is not efficient for free fatty acids. In figures 1.2, 1.3, 1.4, 1.5 and 1.6, the Rfs for neuter fat standards are presented, employing petroleum ether, ethyl ether and acetic acid as mobile phase.

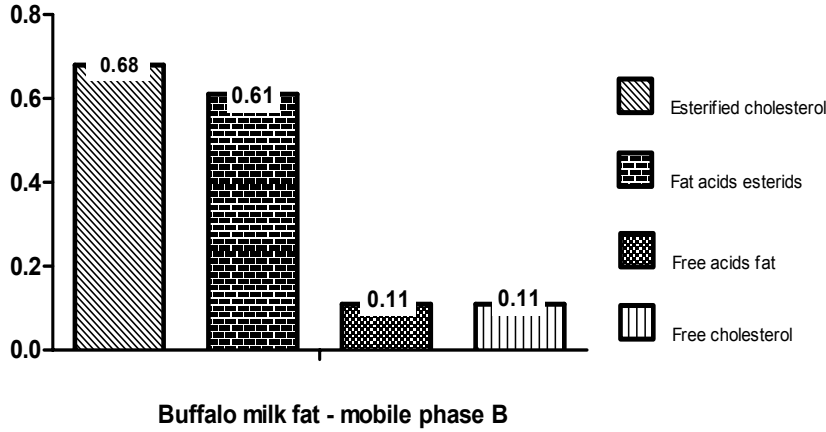

Figure 1.3 Variability of buffalo milk fats through the employment of mobile phase B

The semi-quantitative analysis was conducted through the visual comparison of spot intensity for free cholesterol in analysed samples and the standard of different concentrations applied on the same board. Free cholesterol spots in analysed samples can be observed after 10 minutes, but they are still clearly visible even three hours after iodine vapour exposure.

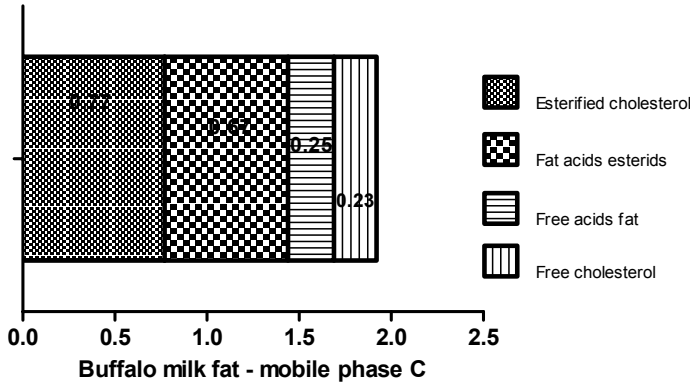

Figure 1.4 Variability of buffalo milk fats through the employment of mobile phase C

As iodine visualisation is not satisfactory, another reactive was employed for visualisation and densiometry. As such, spots were visualised with a copper sulphate/phosphoric acid mixture (3 g copper sulfate and 100 ml phosphoric acid 10% v/v) and heated to 110ºC for 20 minutes. Spot scan was conducted by means of a photodensiometer Camag TLC Scanner 3 to $\lambda = 366$ nm, every hour.

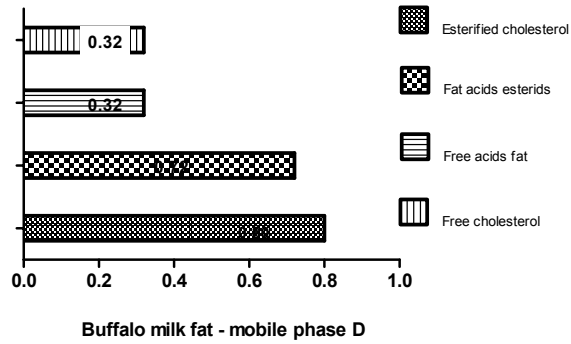

Figure 1.5 Variability of buffalo milk fats through the employment of mobile phase D

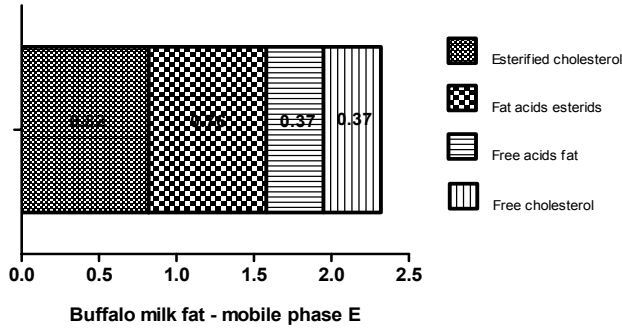

Figure 1.6 Variability of buffalo milk fats through the employment of mobile phase E

CONCLUSIONS

The determination of buffalo milk cholesterol through thin layer chromatography coupled with densitometry is a method that presents a high degree of sensitivity and accuracy. In the case of cholesterol determination through this method, several mobile phases have been employed in order to obtain a satisfactory separation, especially for free cholesterol and free fatty acids. Mobile phase C was selected, which provides a satisfactory separation of the two spots (Rf FFA=0,25 and Rf CL=0,23). Another advantage in the employment of the mobile phase (C) is the separation of neuter fats as narrow bands.

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