

among others, they cause a decrease in the level of anti-arteriosclerosis HDL cholesterol fraction, while at the same time they increase the content of bad LDL cholesterol. They also contribute to an unfavorable functioning of enzymes in the cell membrane, which supports the development of arteriosclerosis and increase the level of insulin in blood and distort the functioning of lymphatic cells [16]. All the research and recently published scientific reports emphasize that the consumption of *trans* fats should be either limited or minimized. A report from the American Institute of Medicine (2005) explicitly states that even a small quantity of *trans* fat in our diet increases the risk of coronary artery disease. Unfortunately, *trans* fatty acids are common in widely available products to such an extent that their complete removal is not possible without an introduction of revolutionary changes in nutritional habits. When establishing recommendations, authors of the abovementioned report took into account the infeasibility of a total elimination of *trans* fats due to the need to eliminate a majority of products from the diet. Thus, the report recommends "a reduction of the consumption of *trans* fats to a minimum while maintaining a wholesome diet" [17;18,19]. The purpose of the present article was to define the composition of fatty acids, and especially the content of *trans* isomers of fatty acids in selected chocolates which are available in the markets throughout the nation (Poland).

2. Materials and Methods:

The chocolate products that were purchased in shopping centers from different producers were included in the study. The selection of chocolate purchase was based on two criteria: the price (three price categories) and the chocolate type (plain chocolates -- Pch and milk chocolates -- Mch). An analysis of the composition of fatty acids in chocolates was performed during the periods of their shelf life as specified by the producers on the packaging.

The fat content in the chocolate products examined was determined with the Weibull-Stoldt and Folch method [20;21]. The purpose of two methods for the isolation of fats was to deduce which of the methods provides a greater repetitiveness of results in terms the quantitative analysis, and how this translated into the composition and content of the individual fatty acids in gas chromatography.

For the analysis, plain chocolate (4--5 g) and milk chocolate (9--10 g) were weighed in a 250 cm³ beaker and 45 ml of boiling distilled water was added with constant stirring to obtain a uniform substance. Furthermore, 55 ml ca. 8 N HCl (2:1) 25% was poured in the solution. Then, 100 ml of boiling water was added, maintaining the boiling state for 30 minutes. The solution was filtered and bathed with water up to the point where the reaction to chlorides disappeared. The wet filtrate was dried at 100 °C for 6 hours, and then extracted for ca. 4 hours in a Soxhlet apparatus. The fat obtained was dried at 100 °C for 2 hours to obtain a solid substance. Isolation of the fat was performed using the Weibull-Stoldt's method for each chocolate in two repetitions and with the Folch's method for comparison.

The next stage was to prepare fatty acid methyl esters. Using a syringe, 20 µl of fat was added to a round-bottom flask and 4 ml of MeOH in 0.5 M NaOH was added to it and warmed in a water bath until the droplets of fat disappeared. Following the fat saponification, 4 ml of 14% BF₃ (boron trifluoride) was added maintaining the solution at the boiling state for 3 minutes. Then 4 ml of hexane was poured maintaining the same boiling temperature for further 3 minutes. Subsequently, anhydrous Na₂SO₄ and brine were added. The methyl esters prepared in the hexane layer were transferred to a 4 ml phial and were marked as methyl esters [22;23;24].

The fatty acids were separated by high resolution gas chromatography (HR-GC) at the following conditions: a capillary column (Chrompack) with the length of 100 m, a diameter of 0.25 mm, film thickness 0.20 µm, a high-efficient stationary phase, initial column temperature of 155°C for 45 minutes followed by an increase in the temperature by 1.5°C per minute up to 210°C which was then maintained for 50 minutes. Hydrogen was a carrier gas, and the linear speed of the gas flow was 26 cm³/s in the temperature of 155°C. The injected sample amount was 1 µl. The chromatograms were registered on ChemStation and processed via HP-Chem programme (Hewlett Packard, Palo Alto, USA). Identification of the eluted peaks of fatty acids and the *trans* isomers of fatty acids was conducted through a comparison with the retention times of the standard fatty acid methyl esters (manufactured by Sigma) and previously published data [28;26;27]. The percentage share of fatty acids was calculated on the basis of the automatic integration of the areas of the elution peaks by software. It was expressed as a percentage share of the individual fatty acids in relation to the total quantity of fatty acids (% m/m).

All the determinations were made in two parallel repetitions.

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