SPECTROPHOTOMETRIC DETERMINATION OF TOTAL STEROLS IN MARGARINE

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Cholesterol as an animal sterol is a precursor of vitamin D3, steroid hormones and bile acids. It is mainly found in food of animal origin. Usually, on food labels you can find information about total and saturated fats but not so often the cholesterol amount. Cutting off butter for health reasons, people use margarine which is cholesterol free but rich in phytosterols. In our research we tried extraction of total sterols from margarine. The protocol started with direct saponification with 1 mol/L methanolic NaOH solution for 15 minutes. After that we added 10 mL deionized water and 1 mL 96% (*v/v*) ethanol and carried out extraction twice with solvent mixture of n-hexane: chloroform (1:1, *v/v*). The moisture was absorbed with anhydrous Na_2SO_4 crystals, filtered and evaporated until dry in room temperature. The formed residue was dissolved in chloroform. Spectrophotometric determination of total sterols was performed with Liebermann – Burchard (LB) method mixing the sample and standard solutions with LB reagent, consisted of acetic anhydride and concentrated sulfuric acid. The dark green product developed after 90 minutes was recorded in 420 nm wavelength. The method showed linearity in analyzed concentration range 0.5 to 0.02 mg/mL (R^2=0.995). Based on LB method, total sterol content in margarine was estimated upon constructed calibration curve and its value was 0.257 mg/mL or 51.4mg/ 100 g sample. The method is simple, cost-effective and sensitive. It is an alternative method to more expensive chromatographic methods.