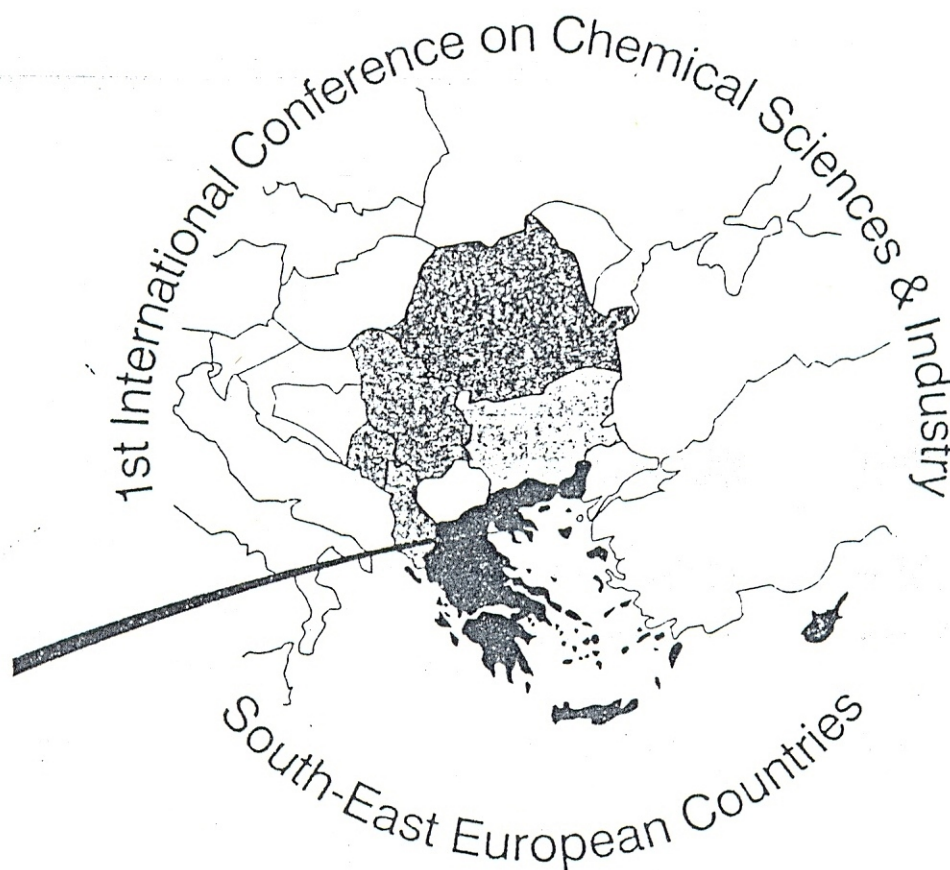


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BOOK OF ABSTRACTS

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DETERMINATION OF A NONENZYMATICALLY PROTEIN - BOUND CARBOHYDRATES IN SERUM BY PHENOL-SULFURIC ACID

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The degree of glycemic control may be performed by analysis of serum glucose content, as well as by determination of glycated haemoglobin (HbA_{1c}) [1] and nonenzymatically protein-bound carbohydrates in serum [2,3].

The extent of nonenzymatic glucosylation of serum protein in diabetic subjects was measured commonly by a chemical procedure using thiobarbituric acid [4] as reagent. But this method is not sensitive and reproductive enough.

In this work a new spectrophotometric method for determination of nonenzymatically serum protein-bound carbohydrates by phenol-sulfuric acid [5] as reagent, was used. All relevant parameters as the conditions of hydrolysis of ketoamine bond between glucose and protein, and formation 5-hydroxymethylfurfural (5-HMF) from glucose, as well as non specific influences on the reaction 5-HMF and phenol-sulfuric acid, were investigated.

It was found that hydrolysis of nonenzymatically bound glucose with 1 mol/dm³ oxalic acid for 4 hours at 100 °C is optimal. For determination released 5-HMF 80% solution of phenol and conc. sulfuric acid in ratio 1:60 were used. A calibration curve was constructed with known concentrations (from 0,25 to 2,50 mmol/dm³) of fructose ($y=0,2395x-0,0099$, $r=0,994$).

The content of protein-bound carbohydrates in serum of diabetics determined by phenol-sulfuric acid (69 ± 24 $\mu\text{mol/dm}^3/\text{g proteina}$) is significantly higher compared to the content obtained by thiobarbituric acid (3.87 ± 1.48 $\mu\text{mol/dm}^3/\text{g proteina}$). To understand the cause of this increase, the reactions between some carbohydrates (mannose, glucose, fructose, arabinose and ramnose) and phenol-sulphuric acid were investigated. For all of sugars, except arabinose, almost equal results were obtained. On the other hand, only fructose with thiobarbituric acid was reacted.

Thus the determination of protein-bound carbohydrates by phenol-sulfuric acid was found more sensitive than spectrophotometric one which use thiobarbituric acid as reagent. More significant correlation with HbA_{1c} content, determined by ion-exchange chromatography, was found. The non-specific influences on the determination was not found. Therefore, the determination of protein-bound carbohydrates by phenol-sulfuric acid can serve as sensitive indicator of the degree hyperglycemia in diabetes.

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