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10-15 July 2023
Târgu Mureș, Romania

BOOK OF ABSTRACTS

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CHEMICAL CHARACTERIZATION OF WINE

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Introduction and objectives: Wine consists of numerous organic and inorganic compounds which determine its quality. Therefore, chemical characterization of wine is very important to be determined in order to understand how the compounds are changing during the winemaking and influencing the taste and aroma.

Materials and methods: The chemical composition of wine was characterized by chromatographic (HPLC-DAD, HPLC-MS, UPLC-QTOF-MS, GC-MS) and spectroscopic methods (AAS, ETAAS, ICP-OES, ICP-MS) as well as with traditional volumetric analytical techniques.

Results and discussion: Determination of organic acids, biogenic amines and polyphenols was performed by reversed phase high-performance liquid chromatography (RP-HPLC) using C18 column for separation of analytes. Detection of compounds was performed with DAD, MS and Q-TOF-MS detectors. Analysis of aroma compounds was performed by gas chromatography coupled to mass spectrometry (GC-MS), while multielement analysis of wines is performed with electrothermal atomic absorption spectroscopy (ETAAS), inductively coupled plasma - optical emission spectrometry (ICP-OES) and inductively coupled plasma - mass spectrometry (ICP-MS). Wines presented complex chemical composition, confirming the stability and importance to the quality.

Conclusions: Advanced analytical techniques were applied for Macedonian red and white wines analysis, including spectroscopic and chromatographic methods, assessing the chemical composition and quality.

Keywords: wine composition, chromatography, spectroscopy.

MIXED-MODE CHROMATOGRAPHY FOR SEPARATION OF PROTEIN DIGESTS

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Introduction and objectives: Protein digestion is usually performed with trypsin, a highly specific serine endoprotease. The bottom-up approach is the most commonly used method for protein identification in proteomics. On the other hand, on-line digestion methods, i.e. direct connection of column with cleaving enzyme (e.g. trypsin) and separation column in a series are only rarely used. Therefore, the aim of this work was to evaluate digestion efficiency of trypsin immobilized enzyme reactors (IMERs) differing in trypsin coverage, connected in series with analytical mixed-mode separation column in LC.

Materials and methods: Two trypsin column reactors with the different trypsin coverage on the bridged ethylene hybrid particles were evaluated. Off-line digestion using trypsin spin columns was performed for comparison of digestion efficiency. Mixed-mode Atlantis Premier BEH C18 AX column combining reversed-phase and anion-exchange properties was used for separation of protein digests. The evaluation of trypsin activity was performed by on-line digestion of N_{α}-benzoyl-L-arginine 4-nitroanilide hydrochloride (L-BAPNA). Various proteins as cytochrome C, enolase, myoglobin etc. were on-line and off-line digested by trypsin.

Results and discussion: Based on the results from BAPNA digestion, optimal chromatographic conditions were applied also for digestion of more complex proteins. Column temperature was 37.0°C, pH of aqueous part of mobile phase (MP) was 8.5, to achieve sufficient trypsin activity. During the initial (digestion) state of analysis, flow rate was decreased, and no organic modifier was present in the MP. Trypsin IMERs were utilized for over 300 injections without any noticeable loss of digestion activity.

Conclusions: By directly connecting the trypsin column to the mixed-mode column in a series, a higher sample throughput can be achieved compared to alternative digestion methods. This approach is uncomplicated and can be adopted in most laboratories without need for LC instrument modifications.

The research was supported by the Czech Science Foundation, Grant No. 20-19655S. The work was partly realized within the cooperation of CEEPUS project No. CIII-RO-0010-17-2223.

Keywords: mixed-mode column, immobilized enzymatic reactor, on-line protein digestion, trypsin