IDENTIFICATION AND CHARACTERIZATION OF DOMESTIC WHEAT VARIETIES BY HPLC AND HPCE

HIGH-PERFORMANCE LIQUID CHROMATHOGRAPHY
AND
HIGH PERFORMANCE CAPILLARY ELECTROPHORESIS

Mirjana Menkovska[1], George Lookhart[2], Desimir Knezevic[3], Marija Sharic[4] and Milisav Ivanoski[5]

[1]PSI Institute of Animal Science, Dpt. of Food Technology and Biotechnology
STS. Cyril and Methodius University, Skopje, R.of Macedonia, mirjanam@unet.com.mk
[2]USDA, GMPRC, Manhattan, College Avenue, 15, Kanzas 66502, USA
[3]Faculty of Agriculture, Lesak, University of Prishtina, R.of Serbia
[4] Faculty of Technology, Dpt. of Grain and Flour, Novi Sad, R. of Serbia
[5] PSI Institute of Agriculture, Sts. Cyril and Methodius University, Skopje, R.of Macedonia

INTRODUCTION

The wheat production, processing, market and other end-users have begun to demand fast and reproducible methods and techniques for varietal identification. High performance liquid chromatography method (HPLC) is widely used for wheat varietal identification and of the other cereals (1, 2, 3), as well as for their characterization trough the gluten proteins (4). This method was used for varietal identification of domestic wheats in combination with the method of acid polyacrylamide gel-elecytrophoresis (A-PAGE) and in wheat quality studies (5, 6), and also in heredity study of gliadin proteins in the early wheat breeding stage (7).

Another technique, which is the simplest and the most often used for the separation of the wheat proteins and for varietal identification, is the free zone capillary electrophoresis (FZCE), which is relatively new technique in the field of cereal hemistry and technology. This technique enables a fast separation of gluten proteins with high resolution (8),

differentiation among the wheat cultivars (9), and their qualitative and quantitative analyses (3).

The aim of this study was to identify wheat cultivars of domestic origin (*Triticum aestivum, L.*), and to characterize the gliadin proteins, using the method of reversed-phase high-performance liquid chromathography (RP-HPLC) and the method of free zone capillary electrophoresis (FZCE).

MATERIALS AND METHOD

Bread wheat cultivars (*Triticum aestivum, L.*) grown in Skopje region, the breeding lines of the Institute of Agriculture in Skopje, were analysed in this study.

RP-HPLC analyses: The method of Lookhart et al (3) was applyed. Gliadins were extracted from 100 mg sample from the well ground wheat kernels, with 50% propanol-1. First albumins and globulins were removed, and also extraction only with propanol was done. In the HPLC system 5-10 µl sample were injected. Analyses were performed at 70^0 at the flow rate of 1 ml/min with multistep gradient system for 26 min. Solution A was 0.1% TFA in water, and solution B was 0.1% TFA in ACN. Detection was performed at 200 nm in the UV region.

FZCE analyses: The gliadins were extracted with 50% propanol-1after removing albumins and globulins, according to the method of Bean and Lookhart (9). Extraction procedure, aparatous description (Beckman PACE 5510/2100), preparation of capillaries and the method used (FZCE) are according to Bean and Lookhart (10). The rejection time of samples was 1 s at pressure of 1.5 psi. All separations were performed at 30 KV and 45 ^{0}C using uncoated silica capillaries (20 cm Ld) x 50 µl inner diameter. Buffer separation was 50 mM iminodiacetic acid (IDA) containing 20% acetonitrile (ACN) and 0.05% hydroxypropyl-methyl-cellulose (HPMC).

RESULTS AND DISCUSSION

By the method of RP-HPLC the gliadin proteins were separated on the basis of the surface hydofobicity and they were characterized by the elution time and the high of the separated picks. Analyses made by this method are shown on the Fig.1. The separation time for gliadin proteins was 22 min and the number of the eluted peaks was up to 25.

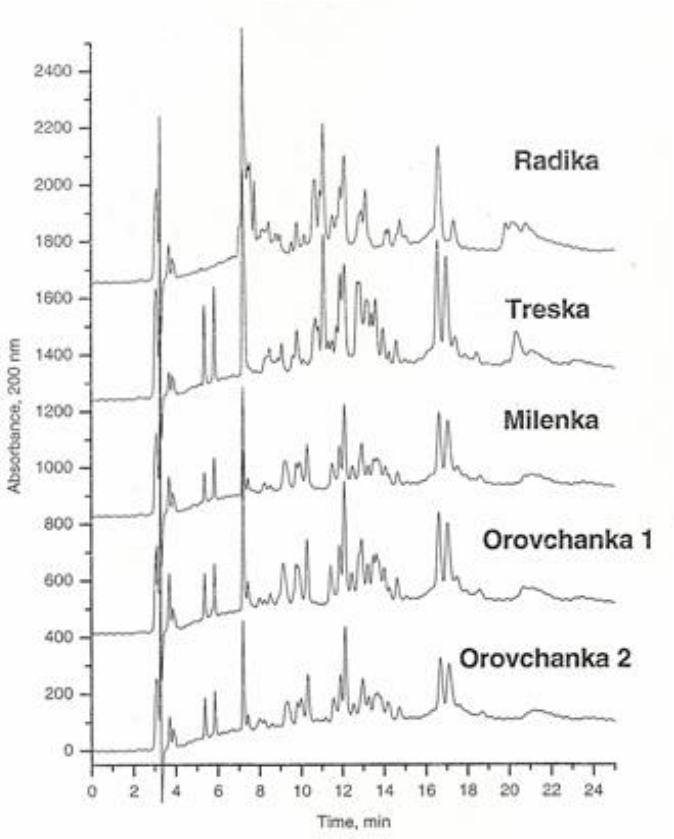

Fig.1 Gliadin extracts of Macedonian bread wheat varieties analysed by RP- HPLC method

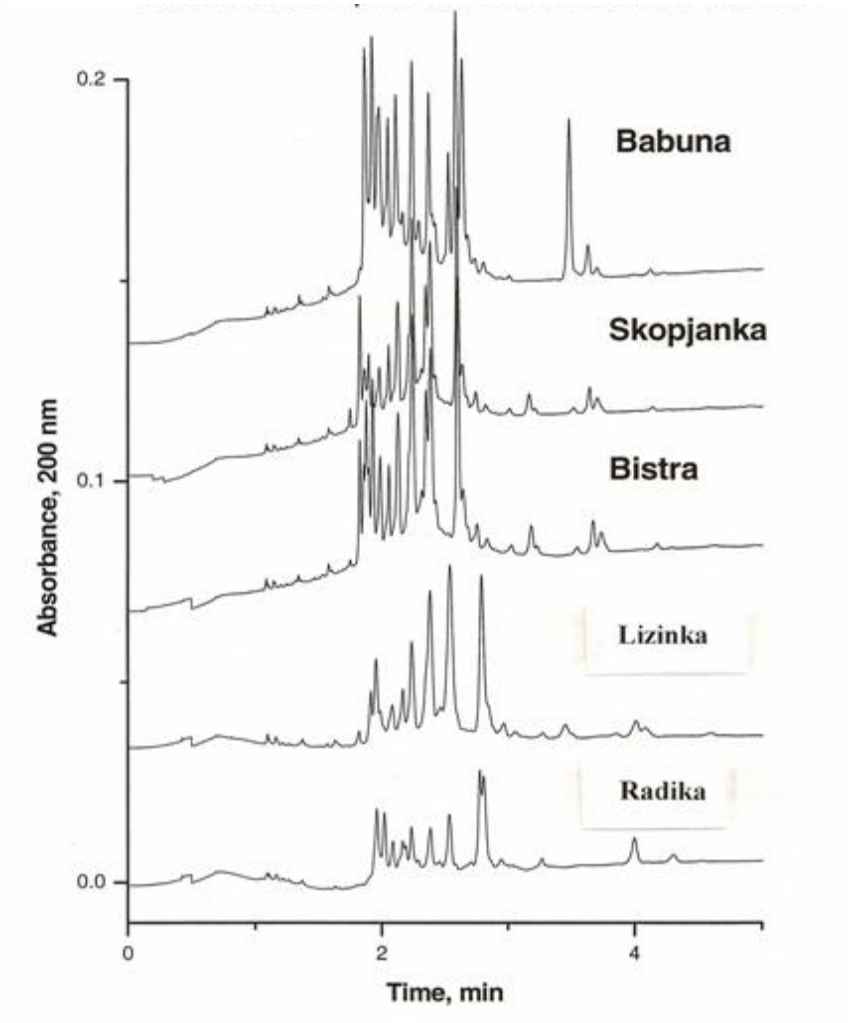

Fig.2 Gliadin extracts of Macedonian bread wheat varieties analysed by HPCE method

With FZCE technique high resolution and reproducibility of gliadin electroforegrams were obtained, which was shown on the Fig. 2. During the four minutes only, gliadins were separated in many peacks (up to 17), comparing to the HPLC method (Fig.1). The wheat varieties which

were analysed by the both identification methods differed within a class, showing similarities and differences in their gliadin composition.

CONCLUSIONS

As conclusion can be stated that by HPLC and FZCE methods identification of domestic wheat varieties and characterization of gluten proteins has been performed, giving *"the finger print"* of the varieties. It was demonstrated their suitability as a fast, equrate and reproducible technique for analysis of gliadin proteins enabling the wheat variety differentiation within a class. This is very important for the production of new wheat varieties, in the early generation of breeding composition of gliadin proteins to be determined and quality properties to be predicted, which will enable breeding of wheat genotypes with desired quality properties. This also enable the applied methods to be included as new standards for wheat quality determination at the wheat market.

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