CHROMATOGRAPHIC SEPARATION OF GLUTENIN WITH HIGH MOLECULAR WEIGHT FROM WHEAT FLOUR

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Abstract

Glutenins with high molecular weight (HMW glutenins) are one of the glutenin's fractions. They play a key role in the formation of the gluten elasticity property and contribute to the formation of large glutenin polymers. The aim of this study was to investigate the effect of solvent type and column temperature on the chromatographic separation of HMW glutenins from wheat flour.

For HMW glutenins extraction, 50% (v/v) ethanol, 1-propanol and isopropanol was used to which Tris-HCl (0.05 mol/L, Ph = 7.5), urea (2 mol/L) and dithioerythritol (1%) were added. The high performance liquid chromatography (HPLC) method (HPLC Agilent Technologies 1260 Infinity, Zorbax 300SB-C3 column) was used for protein separation at a column temperature 40, 45 and 50 ^{0}C. Absorbance measurements were performed at 210 nm and 280 nm.

The effect of the solvents tested on the separation of HMW glutenins was shown by determining the number of observed peaks (proteins) on the chromatogram and calculating the relative concentration of HMW in the total number of glutenins from wheat flour (RC). After the extraction of glutenin proteins with 50% (v/v) ethanol, the highest number of proteins at 210 nm was observed when the column temperature was 45 ^{0}C and 50 ^{0}C (Xsr = 6.17 and RC1 = 17.76% and RC2 = 27.07%) and the lowest number at a column temperature of 40 ^{0}C (Xsr = 4, RC = 13.43%). By extraction with 50% (v/v) 1-propanol, the highest number of proteins was observed at column temperatures of 40 ^{0}C and 45 ^{0}C (Xsr = 5.17 and RC1 = 28.22% and RC2 = 31.70%, respectively) and the lowest number at 50 ^{0}C (Xsr = 4.67, RC = 34.68%) and by extraction with 50% (v/v) isopropanol the highest number of proteins was observed at a column temperature of 50 ^{0}C (Xsr = 7.17, RC = 23.61%) and the lowest number at 45 ^{0}C (Xsr = 5.83, RC = 10.67%). After the extraction of glutenin proteins with 50% (v/v) ethanol and detection at a wavelength of 280 nm, the highest number of proteins was observed at a column temperature of 45 ^{0}C (Xsr = 8.33, RC = 36.49%), and the lowest at 40 ^{0}C (Xsr = 5.50, RC = 32.57%). In the case of protein extraction with 50% (v/v) 1-propanol, the highest number of HMW glutenins was observed at 40 ^{0}C (Xsr = 7.83, RC = 61.62%) and the lowest at 50 ^{0}C (Xsr = 4.67, RC = 39.18%). When extraction was performed with 50% (v/v) isopropanol, the highest number of proteins was observed at a column temperature of 45 °C (Xsr = 7.33, RC = 21.66%), and the lowest number at a column temperature of 40 ^{0}C and 50 ^{0}C (Xsr = 7.00, RC1 = 28.64%, RC2 = 34.22%).

Based on the obtained results it can be seen that the highest number of proteins was observed with 50% (v/v) ethanol (Xsr = 8.33).