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Original scientific paper

Study of the Influence of Solvent and Column Temperature on the Separation Effectiveness of LMW Glutenins by RP-HPLC

Vesna Gojković Cvjetković[1], Radoslav Grujić[2], Željka Marjanović-Balaban[3], Danijela Rajić[1]

[1]*University of East Sarajevo, Faculty of Technology Zvornik, Bosnia and Herzegovina, vesna.gojkovic@yahoo.com*
[2]*State High School of Medical Science, Prijedor, Bosnia and Herzegovina*
[3]*University of Banja Luka, Faculty of Forestry Banja Luka, Bosnia and Herzegovina*

Abstract: Gluten contains two fractions, which are represented in equal amounts. These are soluble gliadins and insoluble glutenins. Glutenin fraction is responsible for the dough and gluten viscoelastic properties. The dominant type of protein in glutenins are LMW glutenins.

The aim of this paper was to examine the influence of solvent type and column temperature on the separation effectiveness of LMW glutenins. Extraction was performed with 50% (v/v) ethanol, 1-propanol and isopropanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added. Separation of LMW glutenins was performed on HPLC Agilent Techologies 1260 Infinity apparatus.

After the extraction with 50% (v/v) ethanol, 1-propanol and isopropanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added, the highest number of proteins was observed after extraction with 50% (v/v) 1-propanol and at a column temperature of 40 °C (Xsr=20, respectively RC=69.56%), and the lowest number at a column temperature of 45 °C (Xsr=14.17, respectively RC=66.42%). The obtained results were read at a wavelength of 210 nm.

After the extraction of LMW glutenins with 50% (v/v) ethanol, 1-propanol and isopropanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and detected at a wavelength of 280 nm, the highest number of proteins was observed after extraction with 50% (v/v) isopropanol and at a column temperature of 50 °C (Xsr=24.17, RC=56.47%) and the lowest number of proteins after extraction with 50% (v/v) ethanol and at a column temperature of 50 °C (Xsr=12.17, RC=56.45%).

Key words: RP-HPLC, C3 column, LMW glutenins.

INTRODUCTION

Glutenins are one of the gluten proteins fraction. These are polymers formed by binding polypeptides to disulfide bonds. They are sparingly soluble proteins and the action of strong reducing agents (β-mercaptoethanol and dithioerythritol) breaks the intermolecular disulfide bonds. As a result, glutenin subunits are released and they are soluble in aqueous alcohol (Urade et al., 2018; Bonilla et a. 2020).

The molecular weight of glutenins varies from 500,000 to 10 and more million daltons (Wieser et al., 2006). Glutenins are divided into two subunits. These are glutenins with high molecular weight (HMW-glutenins) and glutenins with low molecular weight (LMW-glutenins) which give elasticity and viscosity to the dough (Wang et al. 2020; Gao et al., 2017).

The dominant type of glutenin subunits are low molecular weight glutenins (LMW-GS). They make up approximately 60% of total glutenin proteins. Their share in the total gluten protein content is approximately 20% (Wieser and Kieffer, 2001). Glutenins with low molecular weight (LMW-GS) are similar to α+β and γ gliadins in terms of amino acid composition and molecular weight.

They contain the N-terminal region, which consists of repeating units of glutamine and proline. In addition to the N-terminal, they also contain a C-terminal region. This region is homologous to α+β and γ

gliadins. Glutenins with low molecular weight (LMW-GS) contain 8 cysteine residues (Grosch and Wieser, 1999; Wieser, 2003; Huang et al., 2018). Of these, 6 residues are in a position homologous to α+β and γ gliadins. Two additional cysteine residues are unique to glutenins with low molecular weight (LMW-GS) (Wieser, 2007).

Although LMW glutenin subunits are similar to gliadins in primary and secondary structure, they still differ in one very important characteristic. In addition to intramolecular, LMWs also form intermolecular disulfide bonds. These bonds are incorporated into glutenin polymers (Delcour et al., 2012).

The contribution of LMW-GS to dough quality showed complex factors. Studies indicated that both the molecular structures and expression levels of glutenins had important effects on dough quality (Beom et al., 2018). Different types of LMW-GS also affect the dough quality.

Considering the significance of LMW glutenins on dough quality and the fastest and most efficient identification, the aim of this study was to examine the influence of solvent type and column temperature on the efficiency of protein separation by high pressure liquid chromatography with reversed phase.

MATERIAL AND METHOD

Material

Glutenin proteins were extracted from wheat flour type 500 purchased from the market of Bosnia and Herzegovina. 50% (v/v) ethanol, 1-propanol and isopropanol were used for the extraction.

Glutenin extraction

The extraction of glutenin proteins was performed according to a modified method of Wiser et al. (1998) and Gojkovic et al. (2019). First, albumin and globulins (soluble in aqueous and salt solution), then gliadins (soluble in alcohol) were extracted from wheat flour. After these protein fractions were removed, glutenins were extracted. Glutenins were extracted under a nitrogen atmosphere at 60 °C with 50% (v/v) aqueous ethanol (REAHEM, Srbobran), isopropanol (Lach-Ner, Czech Republic) and 1-propanol (Lach-Ner, Czech Republic) in which Tris-HCl (0.05 mol/l, Sigma-Aldrich, United States, pH=7.5), urea (2 mol/l, Sigma Aldrich, Germany) and dithioerythritol (1%, ACROS Organics, Switzerland) were added, at a room temperature, 20 °C.

The extraction was performed twice with 1.0 ml of appropriate solvent each (50% v/v ethanol, 50% v/v 1-propanol and 50% v/v isopropanol). Each time after the extraction solvent was added, the sample was homogenized on a vortex (Advanced Vortex Mixer ZX3, Velp scientifica) for 2 minutes. Then stirring was continued on a magnetic stirrer (Velp scientifica) for 10 minutes. After homogenization was completed, centrifugation of the samples in a centrifuge (Hettich zentrifugen, rotina 380 R) was carried out for 20 minutes at 7000 rpm. The supernatants were made up to 2.0 ml with an appropriate extraction solvent. Samples were filtered through a 0.45 μm membrane filter (RC syringe filters, Filtratech, France) before the analysis began.

Figure 1. shows a chromatogram of glutenins extracted with 50% (v/v) 1-propanol containing Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) and separated at a column temperature of 40 ºC and at a wavelength of 210 nm.

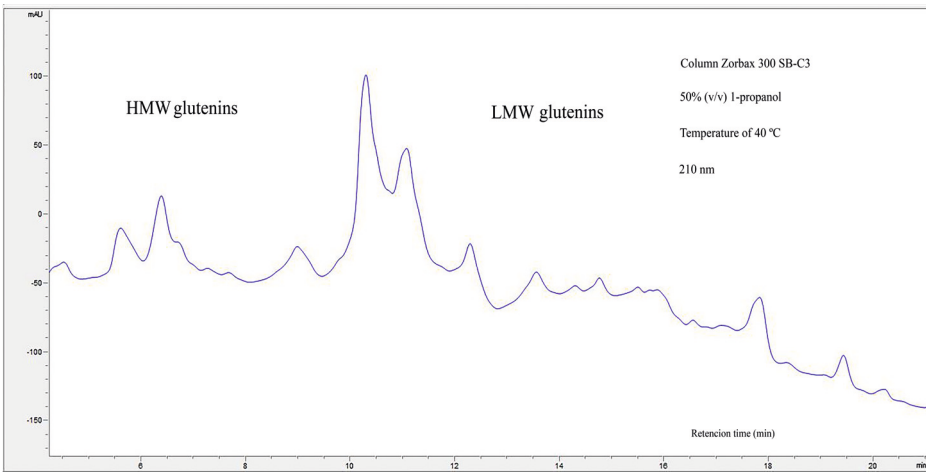

Figure 1. Chromatogram of glutenin proteins

RP-HPLC chromatography

RP-HPLC chromatography was performed on an HPLC Agilent Technologies 1260 Infinity apparatus. The separation of glutenin proteins was performed on a Zorbax 300 SB-C3 column (Agilent Technologies), size 4.6 x 150 mm, with a particle size of 5 µm. The column temperature was 40, 45 and 50 °C. Two mobile phases were used. These are deionized water and 0.1% trifluoroacetic acid (TFA, Acros, France) in acetonitrile (ACN; Biosolve, Chimie, France). The flow rate was 1 ml/min. The injection volume was 70 µl and the detection wavelength was 210 and 280 nm, respectively.

Statistical data processing

Statistical data processing was performed in IBM SPSS, Statistics 26. Descriptive statistical analysis calculated the average value, standard deviation and 95% confidence interval of the average value. Variance analysis of different groups was used to evaluate the effect of column temperature on the number of detected proteins and the relative concentration of LMW glutenins.

RESULTS AND DISCUSSION

Table 1. shows descriptive indicators of the number of LMW glutenin proteins, after extraction with different solvents of the same concentrations (50% v/v ethanol, 50% v/v 1-propanol and 50% v/v isopropanol, in which Tris-HCl 0.05 mol/l, pH=7.5, urea 2 mol/l and dithioerythritol 1%) were added, at different column temperatures (40, 45 and 50 °C), aborbance measured at 210 nm. The number of detected LMW glutenin proteins and their relative concentration is calculated relative to total proteins and total relative concentration.

Table 1. Descriptive indicators for LMW glutenin proteins (solvent 50% v/v ethanol, 1-propanol and isopropanol, RP-HPLC Agilent Technologies 1260 Infinity, Zorbax 300 SB-C3 Agilent column, column size 4.6 x 150 mm, particle sizes 5 µm and column pressure 80 bar, glutenin proteins separation time 21.0 min, measuring the absorbance at 210 nm

Column temperature (°C)		N	Xav	SD	Std. error	95% confidence interval of average		Min	Max
						Lower Bound	Upper bound		
50% (v/v) ethanol	40	6	18.00	0.89	0.36	17.06	18.94	17	19
	45	6	16.33	1.03	0.42	15.25	17.42	15	18
	50	6	16.50	1.05	0.43	15.40	17.60	15	18
50% (v/v) 1-propanol	40	6	20.00	0.63	0.26	19.34	20.66	19	21
	45	6	14.17	1.17	0.48	12.94	15.39	13	16
	50	6	15.50	0.55	0.22	14.93	16.07	15	16
50% (v/v) isopropanol	40	6	18.17	0.75	0.31	17.38	18.96	17	19
	45	6	18.83	1.33	0.54	17.44	20.23	18	21
	50	6	18.67	0.52	0.21	18.12	19.21	18	19
ANOVA (LMW) 50% (v/v) ethanol, F(2.15)=5.11, Sig.=0.020, eta square=10.11/24.94=0.40									
ANOVA (LMW) 50% (v/v) 1-propanol, F(2.15)=81.37, Sig.=0.000, eta square=112.11/122.44=0.92									
ANOVA (LMW) 50% (v/v) isopropanol, F(2.15)=0.83, Sig.=0.454>0.05									

Descriptive analysis showed that the highest number of proteins within the LMW glutenin fraction was obtained after extraction with 50% (v/v) ethanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 40 °C (Xav=18). The lowest number of proteins was obtained at a column temperature of 45 °C (Xav=16.33). As the column temperature increases, the number of proteins decreases, and then slightly increases (Table 1).

Descriptive analysis showed that the highest number of proteins within the LMW glutenin fraction was obtained after extraction with 50% (v/v) 1-propanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 40 °C (Xav=20). The lowest number of proteins was obtained at a column temperature of 45 °C (Xav=14.17). As the column temperature increases, the number of proteins decreases, and then slightly increases (Table 1).

Descriptive analysis showed that the highest number of proteins within the LMW glutenin fraction was obtained after extraction with 50% (v/v) isopropanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 45 °C (Xav=18.83). The lowest number of proteins was obtained at a column temperature of 40 °C (Xav=18.17). As the column temperature increases, the number of proteins increases, and then slightly decreases (Table 1).

Based on the obtained results (Table 1), it can be seen that the highest number of LMW glutenin proteins was obtained by extraction with 50% (v/v) with 1-propanol and chromatographic separation at a column temperature of 40 °C (Xav=20). One-factor analysis of variance examined the effect of column temperature at a constant solvent concentration (50% v/v 1-propanol to which Tris-HCl 0.05 mol/l, pH=7.5, urea 2 mol/l and dithioerythritol 1% were added, glutenin protein separation time and absorbance measurement) on the number of proteins within the LMW glutenin fractions. It was found that there is a statistically significant difference in the number of proteins within the LMW glutenin fractions.

Table 2. shows descriptive indicators of the relative concentration of LMW glutenin proteins, after extraction with different solvents of the same concentrations (50% v/v ethanol, 50% v/v 1-propanol and

50% v/v isopropanol, in which Tris-HCl 0.05 mol/l, pH=7.5, urea 2 mol/l and dithioerythritol 1%) were added, at different column temperatures (40, 45 and 50 °C).

Table 2. Descriptive indicators of the relative concentration of LMW glutenins (solvent 50% v/v ethanol, 1-propanol and isopropanol, RP-HPLC Agilent Technologies 1260 Infinity, Zorbax 300 SB-C3 Agilent column, column dimensions 4.6 x 150 mm, particle sizes 5 µm and column pressure 80 bar, glutenin proteins separation time 21.0 min, measuring the absorbance at 210 nm

Column temperature (°C)		N	Xav	SD	Std. error	95% confidence interval of average		Min	Max
						Lower Bound	Upper bound		
50% (v/v) ethanol	40	6	84.99	1.47	0.60	83.46	86.54	83.48	87.34
	45	6	80.59	2.34	0.96	78.13	83.05	78.00	84.80
	50	6	71.48	1.94	0.79	69.45	73.52	69.75	75.23
50% (v/v) 1-propanol	40	6	69.56	0.74	0.30	68.78	70.34	68.61	70.70
	45	6	66.42	2.49	1.01	63.81	69.03	64.01	70.55
	50	6	63.25	2.28	0.93	60.86	65.65	60.21	66.89
50% (v/v) isopropanol	40	6	79.93	1.72	0.70	78.13	81.74	78.29	83.12
	45	6	88.66	1.93	0.79	86.64	90.69	85.39	91.05
	50	6	75.00	3.18	1.30	71.66	78.34	70.65	80.34
ANOVA (LMW) 50% (v/v) ethanol, F(2.15)=74.89, Sig.=0.000, eta square=569.83/626.90=0.91									
ANOVA (LMW), 50% (v/v) 1-propanol, F(2.15)=15.00, Sig.=0.000, eta square=119.45/179.16=0.67									
ANOVA (LMW) 50% (v/v) isopropanol, F(2.15)=51.33, Sig.=0.000, eta square=574.47/658.41=0.87									

Descriptive analysis showed that the highest relative concentration within the LMW glutenin fraction was obtained after extraction with 50% (v/v) ethanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 40 °C (Xav=84.99%). The lowest relative concentration within the LMW glutenin fraction was obtained at a column temperature of 50 °C (Xav=71.48%). As the column temperature increases, the relative concentration decreases (Table 2).

Descriptive analysis showed that the highest relative concentration within the LMW glutenin fraction was obtained after extraction with 50% (v/v) 1-propanol to which Tris-HCl (0.05 mol/l, pH= 7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 40 °C (Xav=69.56%). The lowest relative concentration within the LMW glutenin fraction was obtained at a column temperature of 50 °C (Xav=63.25%). As the column temperature increases, the relative concentration decreases (Table 2).

Descriptive analysis showed that the highest relative concentration within the LMW glutenin fraction was obtained after extraction with 50% (v/v) isopropanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 45 °C (Xav=88.66%). The lowest relative concentration within the LMW glutenin fraction was obtained at a column temperature of 50 °C (Xav=75.00%). As the column temperature increases, the relative concentration increases and then decreases.

Based on the obtained results (Table 2), it can be seen that the highest relative concentration of LMW glutenin proteins was obtained by extraction with 50% (v/v) isopropanol and chromatographic separation at a column temperature of 45 °C (RC=88.66%). One-factor analysis of variance examined the effect of column temperature at a constant solvent concentration (50% v/v isopropanol to which Tris-HCl 0.05 mol/l, pH=7.5, urea 2 mol/l and dithioerythritol 1% were added, glutenin protein separation time and ab-

sorbance measurement) on relative concentration of the LMW glutenin fractions. It was found that there is a statistically significant difference in the relative concentrations of LMW glutenin fractions.

Table 3. shows descriptive indicators of the number of LMW glutenin proteins, after extraction with different solvents of the same concentrations (50% v/v ethanol, 50% v/v 1-propanol and 50% v/v isopropanol, in which Tris-HCl 0.05 mol/l, pH=7.5, urea 2 mol/l and dithioerythritol 1%) were added, at different column temperatures (40, 45 and 50 °C), aborbance measured at 280 nm.

Table 3. Descriptive indicators for LMW glutenin proteins (solvent 50% v/v ethanol, 1-propanol and isopropanol, RP-HPLC Agilent Technologies 1260 Infinity, Zorbax 300 SB-C3 Agilent column, column size 4.6 x 150 mm, particle sizes 5 µm and column pressure 80 bar, glutenin proteins separation time 21.0 min, measuring the absorbance at 280 nm

Column temperature (°C)		N	Xav	SD	Std. error	95% confidence interval of average		Min	Max
						Lower Bound	Upper bound		
50% (v/v) ethanol	40	6	15.00	1.26	0.52	13,.67	16.33	14	17
	45	6	16.33	1.03	0.42	15.25	17.42	15	18
	50	6	12.17	0.75	0.31	11.38	12.96	11	13
50% (v/v) 1-propanol	40	6	17.33	0.82	0.33	16.48	18.19	16	18
	45	6	14.00	0.63	0.26	13.34	14.66	13	15
	50	6	15.50	1.52	0.62	13.91	17.09	13	17
50% (v/v) isopropanol	40	6	19.83	0.41	0.17	19.40	20.26	19	20
	45	6	16.67	1.37	0.56	15.23	18.10	14	18
	50	6	24.17	0.75	0.31	23.38	24.96	23	25
ANOVA (LMW), 50% (v/v) ethanol, F=(2.15)=25.21, Sig.=0.000, eta square=54.33/70.50=0.77									
ANOVA (LMW), 50% (v/v) 1-propanol, F(2.15)=14.90, Sig.=0.000, eta square=33.44/50.28=0.66									
ANOVA (LMW), 50% (v/v) isopropanol, F(2.15)=98.14, Sig.=0.000, eta square=170.11/183.11=0.93									

Descriptive analysis showed that the highest number of proteins within the LMW glutenin fraction was obtained after extraction with 50% (v/v) ethanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 45 °C (Xav=16.33). The lowest number of proteins within the LMW glutenin fraction was obtained at a column temperature of 50 °C (Xav=12.17). As the temperature of the column increases, the number of proteins increases and then decreases (Table 3).

Descriptive analysis showed that the highest number of proteins within the LMW glutenin fraction was obtained after extraction with 50% (v/v) 1-propanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 40 °C (Xav=17.33). The lowest number of proteins within the LMW glutenin fraction was obtained at a column temperature of 45 °C (Xav=14). As the temperature of the column increases, the number of proteins decreases and then increases (Table 3).

Descriptive analysis showed that the highest number of proteins within the LMW glutenin fraction was obtained after extraction with 50% (v/v) isopropanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 50 °C (Xav=24.17). The lowest number of proteins within the LMW glutenin fraction was obtained at a column temperature of 45 °C (Xav=16.67). As the temperature of the column increases, the number of proteins decreases and then increases (Table 3).

Based on the obtained results (Table 3), it can be seen that the highest number of LMW glutenin proteins was obtained by extraction with 50% (v/v) isopropanol and chromatographic separation at a column temperature of 50 °C (Xav=20.17), at wavelength of 280 nm. One-factor analysis of variance examined the effect of column temperature at constant solvent concentration (50% v/v isopropanol to which Tris-HCl 0.05 mol/l, pH=7.5, urea 2 mol/l and dithioerythritol 1%, separation time glutenin protein and absorbance measurement) to the number of proteins within the LMW glutenin fraction. It was found that there is a statistically significant difference in the number of proteins.

Table 4. shows descriptive indicators of the relative concentration of LMW glutenins, after extraction with different solvents (50% v/v ethanol, 50% v/v 1-propanol and 50% v/v isopropanol to which Tris-HCl 0.05 mol/l, pH=7.5, urea 2 mol/l and dithioerythritol 1% were added, at a different column temperatures (40, 45 and 50 °C).

Table 4. Descriptive indicators of the relative concentration of LMW glutenins (solvent 50% v/v ethanol, 1-propanol and isopropanol, RP-HPLC Agilent Technologies 1260 Infinity, Zorbax 300 SB-C3 Agilent column, column size 4.6 x 150 mm, particle sizes 5 µm and a column pressure 80 bar, glutenin proteins separation time 21.0 min, measuring the absorbance at 280 nm

Column temperature (°C)		N	Xav	SD	Std. error	95% confidence interval of average		Min	Max
						Lower Bound	Upper bound		
50% (v/v) ethanol	40	6	64.29	1.21	0.49	63.02	65.56	62.88	66.35
	45	6	60.89	2.59	1.06	58.17	63.60	56.86	64.18
	50	6	56.45	2.56	1.05	53.76	59.14	53.48	61.14
50% (v/v) 1-propanol	40	6	37.75	3.32	1.36	34.26	41.24	34.23	43.62
	45	6	62.09	2.88	1.17	59.07	65.11	56.86	64.32
	50	6	52.56	2.82	1.15	49.61	55.52	48.48	55.14
50% (v/v) isopropanol	40	6	59.81	2.20	0.90	57.50	62.12	56.47	62.96
	45	6	69.86	4.24	1.73	65.41	74.30	63.76	75.30
	50	6	56.47	3.66	1.50	52.63	60.32	52.43	63.12
ANOVA (LMW), 50% (v/v) ethanol, F=(2.15)=18.86, Sig.=0.000, eta square=185.31/258.99=0.71									
ANOVA (LMW), 50% (v/v) 1-propanol, F(2.15)=99.21, Sig.=0.000, eta square=1804.99/1941.45=0.93									
ANOVA (LMW), 50% (v/v) isopropanol, F(2.15)=24.12, Sig.=0.000, eta square=582.32/763.38=0.76									

Descriptive analysis showed that the highest relative concentration within the LMW glutenin fraction was obtained after extraction with 50% (v/v) ethanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 40 °C (Xav=64.29%). The lowest relative concentration within the LMW glutenin fraction was obtained at a column temperature of 50 °C (Xav=56.45%). As the column temperature increases, the relative concentration of LMW glutenin decreases (Table 4).

Descriptive analysis showed that the highest relative concentration within the LMW glutenin fraction was obtained after extraction with 50% (v/v) 1-propanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/ l) and dithioerythritol (1%) were added and at a column temperature of 45 °C (Xav=62.09%). The lowest relative concentration within the LMW glutenin fraction was obtained at a column temperature of 40 °C (Xav=37.75%). As the column temperature increases, the relative concentration of LMW glutenin increases and then decreases (Table 4).

Descriptive analysis showed that the highest relative concentration within the LMW glutenin fraction was obtained after extraction with 50% (v/v) isopropanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and at a column temperature of 45 °C (Xav=69.86%). The lowest relative concentration within the LMW glutenin fraction was obtained at a column temperature of 50 °C (Xav=56.47%). As the column temperature increases, the relative concentration of LMW glutenin increases and then decreases (Table 4).

Based on the obtained results (Table 4), it can be seen that the highest relative concentrations of LMW glutenin proteins was obtained by extraction with 50% (v/v) isopropanol and chromatographic separation at a column temperature of 45 °C (RC=69.86%), at wavelength of 280 nm. One-factor analysis of variance examined the effect of column temperature at constant solvent concentration (50% v/v isopropanol to which Tris-HCl 0.05 mol/l, pH=7.5, urea 2 mol/l and dithioerythritol 1%, separation time glutenin protein and absorbance measurement) to the relatice concentrations of LMW glutenin fractions. It was found that there is a statistically significant difference in the number of proteins.

Based on the obtained results (Table 1 and Table 3), it can be seen that a higher number of proteins was obtained by separation at a wavelength of 280 nm (Xav=24.17), than at a wavelength of 210 nm (Xav=20), but the separation at 210 nm is better, peaks are sharper and it is specific for peptide bond.

Wieser (2000) determined gluten proteins qualitatively and quantitatively in the framework of comparative studies of different wheat varieties. Glutenins were extracted with 50% (v/v) 1-propanol and dithioerythritol (DTE). The detection wavelength was 210 nm. Based on the obtained results, the retention time of LMW glutenin was > 27.

Horvat et al. (2006) analyzed gluten proteins in wheat by RP-HPLC chromatography. Different wheat varieties were used for protein extraction. The column temperature was 50°C and the detection wavelength was 210 nm. Based on the obtained results, retention time for LMW glutenins was 15-30 minutes (16-19 subunits).

Qian et al. (2008) characterized wheat gluten protein by high-pressure liquid chromatography (HPLC) and MALDI TOF mass spectrometry. The column temperature was 60 °C and the detection wavelength was 210 nm. Based on the obtained results, LMW glutenins identified 22 peaks.

Scherf (2016) investigated how the method of preparation of gliadin, glutenin and gluten from wheat starch samples affects their content when determining by high-pressure reverse phase liquid chromatography (RP-HPLC) and enzyme-linked immunosorbent assay (ELISA). Glutenins were extracted with 50% (v/v) 1-propanol containing Tris-HCl (pH=7.5), 0.06 mol/l (v/v) dithioerythritol and 2 mol/l urea. Samples were analyzed by high pressure reverse phase liquid chromatography (RP-HPLC), at a column temperature of 60 °C and at a wavelength of 210 nm. Separation of glutenin proteins was performed for 22 minutes.

Figure 2. shows selected proteins of LMW glutenins. They extracted with 50% (v/v) 1-propanol containing Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) and separated at a column Zorbax 300 SB-C3, Agilent, dimensions 4.6 x 150 mm, particle size 5µm, at a column temperature of 40 ºC and a pressure of 80 bar. Glutenin protein separation time was 21.0 min and at a wavelength of 210 nm.

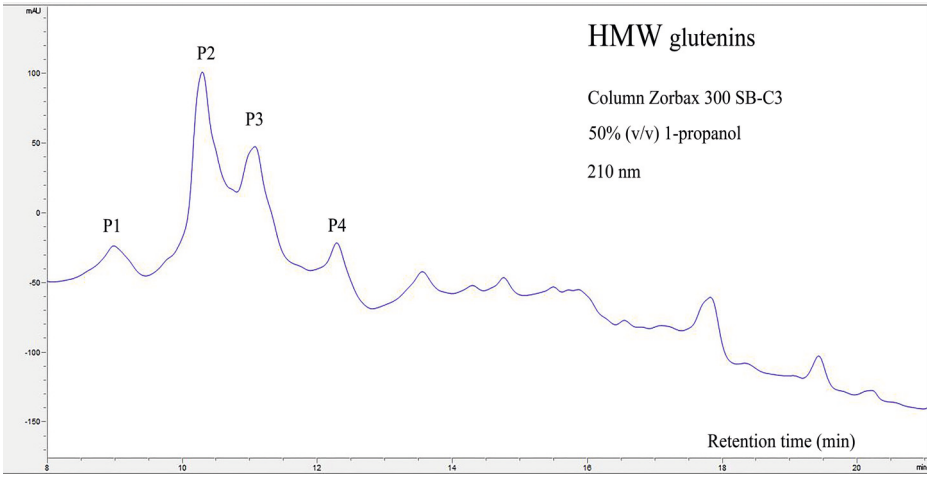

Figure 2. Chromatogram of selected proteins of LMW glutenins

Table 5. shows average values of retention time and relative concentration of selected proteins of LMW glutenins.

Table 5. Average values of retention time and relative concentration of selected LMW glutenins

Proteins	Retention time ± SD	Relative concentration ± SD
P1	8.94 ± 0.10	20.94 ± 0.95
P2	10.29 ± 0.15	26.01 ± 1.20
P3	11.08 ± 0.12	6.84 ± 0.55
P4	12.28 ± 0.08	4.25 ± 0.61

Based on the obtained results it can be seen that repeatability of these proteins is good, the standard deviations is small. Six repetitions were done.

In this work, the influence of solvent type was investigated (50% v/v ethanol, 1-propanol and isopropanol) to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added and column temperature on the separation efficiency and identification of LMW glutenin, in two wavelengths. Separation was performed on column C3. The most effective solvent was 50% (v/v) 1-propanol and a column temperature of 40 °C (Xav=20). The wavelength of 210 nm is more specific because it indicates the presence of a peptide bond. The results obtained in this paper are in agreement with the authors Horvat et al. (2006), Qian et al. (2008) and Scherf (2016).

CONCLUSION

After the LMW glutenins were chromatographically separated on an HPLC chromatograph, and after extraction with different solvents and at different column temperatures, the following conclusions were reached.

After extraction of LMW glutenin with 50% (v/v) ethanol, 1-propanol and isopropanol to which Tris-HCl (0.05 mol/l, pH=7.5), urea (2 mol/l) and dithioerythritol (1%) were added, at different column temperatures (40, 45 and 50 °C) and by measuring the absorbance at 210 nm, the highest number of proteins was obtained by extraction of 50% (v/v) with 1-propanol and at a column temperature of 40 °C (Xav=20, respectively RC=69.56%), and the lowest number at a column temperature of 45 °C (Xav=14.17, respec-

tively RC=66.42%). When measuring the absorbance at 280 nm, and by extraction and separation under the same conditions as previously mentioned, the highest number of proteins was obtained by extraction of 50% (v/v) isopropanol and at a column temperature of 50 °C (Xav=24.17, respectively RC=56.47%), and the lowest number by extraction with 50% (v/v) ethanol and at a column temperature of 50 °C (Xav=12.17, respectively RC=56.45%).

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