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SCIENTIFIC PAPER

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LAB-ON-A-CHIP METHOD UNCERTAINTIES IN DETERMINATION OF HIGH-MOLECULAR--WEIGHT GLUTENIN SUBUNITS

Polymeric wheat endosperm proteins, especially the high-molecular-weight glutenin subunits (HMW-GS), are probably the most interesting protein fraction giving the essential information about the bread-making quality of wheat flour. A relatively new method that shows great potential for a fast, reliable and automatable analysis of protein purity, sizing and quantification is microfluidic or Lab-on-a-Chip (LoaC) capillary electrophoresis. This aim of this work was to explore the possibilities of implementation of LoaC method to analysis of protein samples isolated from a Serbian common wheat variety, emphasizing the steps that might bring uncertainties and affect reproducibility of obtained glutenin subunits quantitation results. A good resolution of protein bands in a molecular weight range of 14.0 to 220.0 kDa was achieved. The reproducibility of HMW-GS sizing and quantitation were good, with the average coefficient of variation values of 1.2 and 12.2%. The ratio of HMW-GS to low-molecular-weight glutenin subunits (LMW-GS) was about 20%. The investigation ruled out influences of the extract solution addition and the buffer addition steps of the applied method, as well as the individual chip influence on GS quantitation results. However, there was statistically significant difference between HMW-GS quantitation results of multi-step and one-step extraction procedures applied prior to glutenin subunits extraction step.

Keywords: wheat, glutenin subunits, Lab-on-a-Chip, Arija wheat variety, LoaC Lab-on-a-Chip.

Wheat gluten has a major effect on the end-use quality of baking industry products, since it is responsible for the visco-elastic properties of the dough. This protein macromolecule is composed of two components, gliadins and glutenins. Gliadins are viscosous and affect the extensibility of dough, whereas glutenins are responsible for the dough elasticity [1]. Also, gliadins are single-chain polypeptides with molecular weights (MW) between 30,000 and 80,000 Da, whereas glutenins are multichain polypeptides with MW ranging from 80,000 to several millions Da [2,3]. Glutenins could further be divided into two groups, high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW--GS). It is a well-known fact, that diverse HMW-GS are correlated with bread-making quality [4], for instance, 5+10 depict good quality and 2+12, 4+12 subunits are connected with lack of dough strength. Hou *et al.* [5] confirmed that a positive correlation exists between HMW-GS content and rheological properties of wheat dough, whereas different scientists emphasize significant influence of HMW-GS quantity in prediction of dough or gluten strength [6,7]. MacRitchie [8] also showed that the glutenin:gliadin (Glu:Gli) ratio shows considerable influence on dough and pan bread loaf quality.

Electrophoresis and liquid chromatography are techniques that have been commonly applied for cereal proteins separation [9]. The most common electrophoretic methods for examination of cereal proteins is sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE). However, this form has several disadvantages. For instance, SDS-PAGE is time-consuming and includes a number of necessary manual steps, such as staining, destaining, imaging, analyzing [10]. Quantification can also be difficult [11]

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and one of the used chemical is acrylamide, a potential neurotoxin.

The new, promising, fast electrophoretic technique for protein examinations is a microfluidic or Lab-on-a-Chip (LoaC) method, which allows the integration of electrophoretic separation, staining, destaining, and fluorescence detection into a single process which can be combined with data analysis. This new technique is comparable to time consuming SDS-PAGE stained with standard Coomassie in sensitivity, sizing accuracy and reproducibility [12]. However, the sizing accuracy of SDS-PAGE and chip-based analysis depend on the protein characteristics and may therefore vary for particular proteins. Some proteins may not migrate according to their molecular weight [12]. In general, the sizing reproducibility of the LoaC method is excellent, commonly achieving a sizing reproducibility of 5% or better [13]. In addition to sizing, the chip-based assay provides means for absolute protein quantitation based on user-defined standards with known protein concentration or relative protein quantification based on internal standards [12]. Absolute quantitation can be obtained by using a calibration curve generated with the same protein. A protein calibration feature in the software of the chip-based analysis system automatically generates a protein calibration curve to determine the absolute concentration of actual samples within the same chip. Absolute protein concentrations as well as protein purity and size are determined in a single experiment [12]. Relative protein concentrations are determined using a one-point calibration, comparing the peak area of the protein of interest with the peak area of the upper marker, which is used as an internal standard in each sample, with known protein concentrations [12]. Internal standard based quantitation is used for correction of different injection efficiencies due to varying salt concentrations and permits determination of the relative concentration independently of the sample matrix. The relative concentration depends on the staining efficiency and can vary from protein to protein [12]. Each of the commonly used total protein quantitation assay methods, such as the Lowry or Bradford assays, as well as the SDS-PAGE method, which allows the quantitation of individual proteins within a sample, exhibit some degree of variation in staining efficiency when assaying different proteins. The quantitation accuracy and reproducibility of the chip-based bovine serum albumin protein analysis are comparable to that achieved with the batch-based Lowry and Bradford assays, and better than the ones achieved by using SDS-PAGE [12]. Reproducibility of the relative quantitation of different proteins in a model mixture with LoaC method, expressed as relative standard deviation (coefficient of variation, *C.V.*) was below 30% [13]. Agilent specifies an area reproducibility of 20% (*C.V.*) relative to the upper marker. There is some area variability between instruments and chip runs due to either slightly different optical setups or differences in electrokinetic sample injection, and the average area reproducibility for an individual instrument is 15.1%. Therefore, area differences between instruments or individual chip runs are accounted for and do not affect protein quantitation [14]. However, all these findings are basically determined on model systems and they are still by no means confirmed in a real matrix.

Several authors used LoaC method for identification and quantification HMW-GS in different wheat varieties [15-18], whereas Baláz *et al.* [19] confirmed that influence of environmental conditions on quantity of wheat protein subunits could be monitored using this method. Aditionally, Baláz *et al.* [20] showed that this technique allows good separation and quantification of wheat albumins and globulins, whereas for segregation of wheat gliadin it was not adequate. Chanvrier *et al.* [21] followed the polymerization of protein of wheat gluten under processing such as extrusion, while Maforimbo *et al.* [22] studied the interaction of glutenin subunits and soy proteins by LoaC method. Furthermore, molecular weight and concentration of different compounds which are involved in biochemical processes such as nicotinamide adenine dinucleotide phosphate NAD(P)+ isolated from pea, soybean, and wheat proteins [23] and Kunitz trypsin inhibitor in soybean varieties [24] can be determined by the LoaC method.

This aim of this work was to explore the possibilities of application of this novel method for analysis of glutenin subunits, especially HMW-GS, isolated from a Serbian common wheat variety Arija. Results of the chip-based protein sizing analysis were provided, as well as the concentrations of the glutenin subunits within Arija wheat variety flour sample (in ppm), obtained using a one-point calibration. Wheat flour samples were subjected to two different extraction procedures, and relative quantitation results were compared. The first multi-step extraction procedure, which was applied in order to remove albumin, globulin and gliadin protein, was compared to one-step extraction procedure removing only gliadin proteins. Special interest was directed to determination of the overall reproducibility of relative quantitation of glutenin subunits on this microchip platform in order to emphasize the steps that might bring uncertainties

and affect reproducibility of obtained results, such as the individual chip influence, an extract solution addition step, or a buffer addition step. The reproducibility of sizing and relative quantitation of glutenin subunits on microchip were evaluated by examining six replicates of each sample on three different chips. The limit of detection (*LOD*) and the limit of quantitation (*LOQ*) were obtained for a signal-to-noise ratio (*S*/*N*) of 3 and 10, respectively.

EXPERIMENTAL

The investigated sample of Arija wheat vairety with HMW-GS subunits in pairs 7+8, 5+10 and 2+12, kindly supplied by NS SEME Novi Sad, Serbia, was milled by MLU 202 mill Bühler (Switzerland) to 60% flour yield. Three samples, each about 30 mg, were taken for protein analysis (Figure 1). In order to remove albumin, globulin and gliadin proteins, two obtained flour samples (I-1and I-2 in Figure 1) were subjected to three consecutive extraction processes, with three different solvents: deionized water, 2% salt solution and 70% ethanol solution. Each time a volume of 300 µl of the solvent was mixed with the flour sample on a vortex mixer for 10 s, and after 24 h extraction period at room temperature, centrifuged for 20 min at 14500 r/min. Third flour sample (Sample II in Figure 1) was extracted only with 70% ethanol solution to remove gliadin proteins. The full range of glutenin subunits was then extracted with an extract solution (2% SDS solution containing 5% *β*-mercaptoethanol). A volume of 350 µl of the extract solution was added and subsequently heated for 5 min to 100 °C. Glutenin subunits of the first and the third samples were extracted with the same SDS solution (Sol-1 in Figure 1), whereas for the second sample the new portion of SDS solution was prepared and used (Sol-2 in Figure 1). A final solution to be applied on Agilent LabChips was prepared by mixing a volume of 4 µl of the clarified sample extract with 2 µl of Agilent sample buffer and 84 µl of deionized water. In order to rule out the influence of the buffer addition step on the overall reproducibility, for sample I-1 the same procedure was applied in triplicate, and three final glutenin subunits extract solutions, labeled I-1a, I-1b, I-1c (Figure 1) were formed. The last two final samples, labeled I-2 and II (Figure 1) were obtained from the flour samples 2 and 3, respectively. Three Agilent LabChips were used for the analysis. The sample I-1a was applied to four of the 10 sample wells on the same Agilent LabChip, and on two other chips, while the other four samples (I-1b, I-1c, I-2 and II) were applied on all three chips (Figure 1).

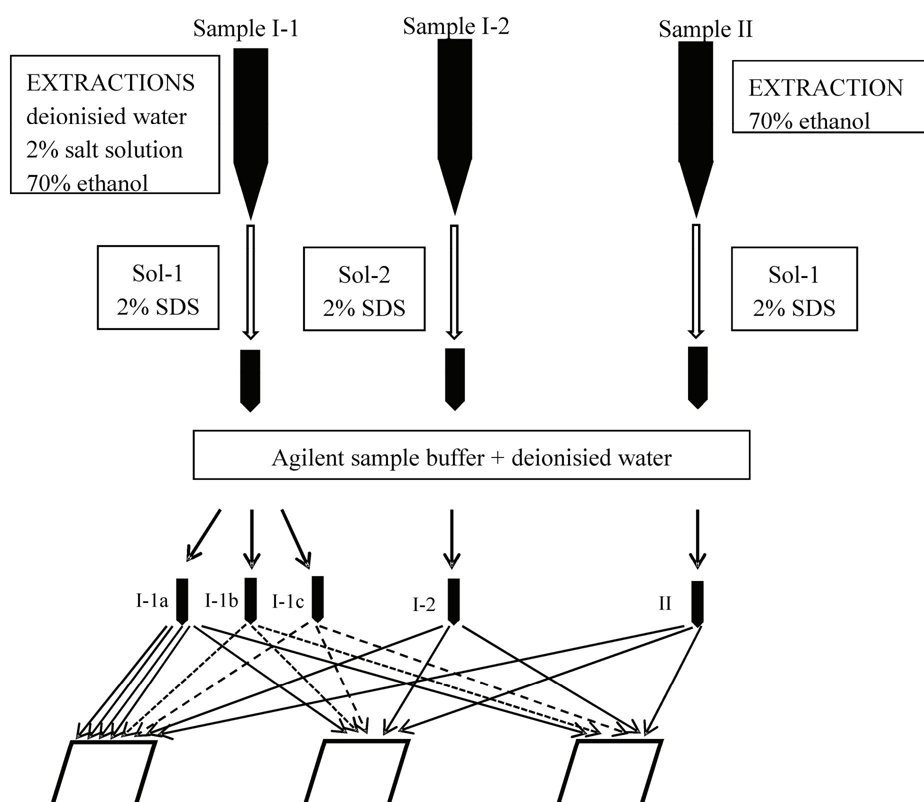

Figure 1. Workflow of wheat flour samples extractions and the extracts applications on the Agilent LabChips.

Extracted glutenin subunits as described above were analysed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) in combination with the Protein 230 Plus LabChip kit and the dedicated Protein 230 software assay on 2100 expert software. Each sample contained internal standard comprising of upper marker of 240 kDa and lower marker of 4.5 kDa (originated from the buffer). Each chip included a ladder comprising of reference proteins of 15, 26, 46, 63, 95 and 150 kDa, plus the upper and the lower markers (240 and 4.5 kDa), against which electrophoretic mobilities were compared for each analysis. The relative concentration of glutenin subunits were determined using a one-point calibration to the upper marker (60 ng/μl) included in each sample and calculated in ppm using measured flour weight of each flour sample and volume used for their extraction (350 μl).

RESULTS AND DISCUSSION

Protein sizing with the chip-based protein analysis system was performed by running the protein sizing standard on each chip from a designated well. Following the analysis of this sizing standard, the software generated a standard curve of the measured migration time versus the known molecular weight of each standard protein which was used to determine the size of each of the proteins detected within the sample [12]. Internal standards, the lower and upper marker, were included in each sample.

The results of the sizing analysis, the sizing accuracy and reproducibility of the chip-based protein assay, based on six replicated runs on three different chips, are presented in Table 1. The molecular weights are shown in kDa.

Good resolution of the glutenin subunits bands in a molecular weight range from 14.0 to 220 kDa was achieved when the chips and protein isolate samples, obtained after the multi-step extraction procedure were prepared according to the suggested protocol. The average sizing reproducibility (relative standard deviation or the coefficient of variation) was 1.32%; Baláz *et al*. [19] gained relative standard deviation of wheat protein peaks in the range from 0.15 (peak 14.1 kDa) to 4.89% (220.1 kDa). Six protein bands corresponding to HMW-GS were in a molecular weight range from 100 to 220 kDa which is in accordance with findings Marchetti-Deschmann *et al.* [18] and Baláz *et al*. [19]. The results reported by Baláz *et al*. [19] who used the same methodology were also used as the base for identification of HMW-GS. They occupy the top third of the patterns on the gel-like image, above black line, showing the analysis of the protein sample (Figure 2). The lanes 1 and 2 show multi-step extractions, lane 3 represents the one-step extraction, whereas lane 4 depicts the pattern of molecular weight standards (Figure 2). It is obvious that color intensity of protein bands in lane 3 (one-step extraction) is higher than in lane 1 and 2 (multi-step extractions). Also, these six protein bands are labeled on electropherograms (Figure 3) with their mean sizes of molecular weight (kDa). The red line and blue line depict electropherograms of the multi-step extractions, whereas the green line represents the electropherogram of the one-step extraction with ethanol. Areas of the peaks of identified glutenin subunits and upper marker under the green electropherogram are higher than areas of the peaks of identified glutenin subunits and upper marker of the red and blue line presented electropherograms. The differences among electropherograms are effect of applied multi-step and one-step extractions and confirmed the working hypotheses that using of different extractions will influence on quantity of examined glutenin subunits. Literature values of the sizing reproducibility (relative standard deviation) of the chip-based protein assay for the inter and intra-chip comparison of wheat proteins of three single wheat varieties were not higher than 2.2% [18] compared with relative standard deviation of various glycosylated proteins in the range from 1.6 to 3.9% [25].

Table 1. Glutenin subunits chip-based sizing analysis results of Arija wheat variety flour samples

Mean size ±SD, kDa	*C.V.* / %
14.0±0.2	1.53
15.6±0.5	2.96
37.5±0.4	1.00
41.5±0.5	1.12
44.5±0.4	0.99
48.7±1.0	2.09
53.8±0.6	1.15
59.9±0.8	1.34
62.1±0.7	1.12
71.6±0.9	1.32
97.9±0.9	0.90
122.7±2.0	1.60
132.1±1.0	0.77
140.0±1.0	0.75
149.5±1.8	1.19
187.2±2.2	1.16
219.7±3.6	1.63
GS, Average *C.V.*	1.32
HMW-GS, Average *C.V.*	1.18

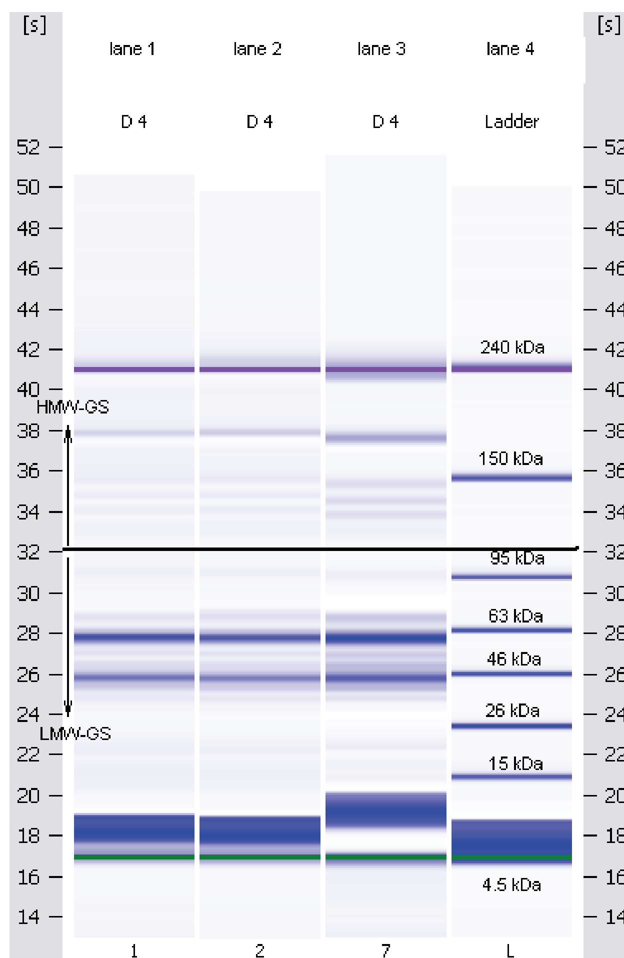

Figure 2. The gel-like image from the Lab-on-a-chip capillary electrophoresis of Arija wheat-flour proteins. The HMW subunits of glutenin occupy the top third of the patterns (above black line). The lane 1 and 2 show multi-step extractions, lane 3 represents one-step extraction whereas lane 4 depicts the pattern of molecular weight standards.

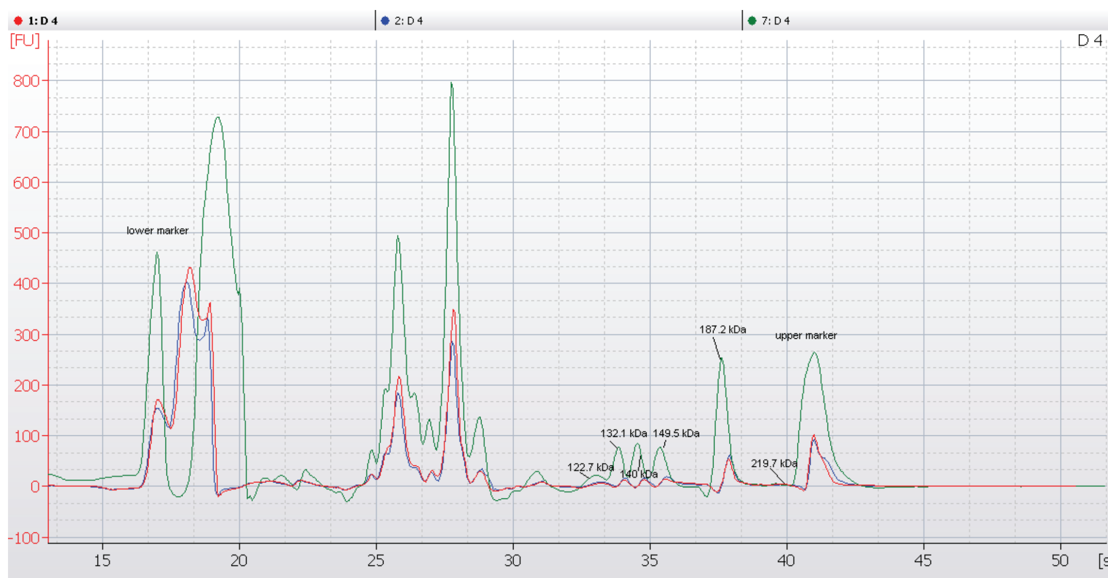

Figure 3. The electropherograms of two multi-step extractions and one-step extraction from the Lab-on-a-chip capillary electrophoresis of Arija wheat-flour. The red line and blue line depict multi-step extraction, whereas the green line represents one-step extraction with ethanol.

The limit of detection (*LOD*) and the limit of quantitation (*LOQ*) were obtained for a signal-to-noise ratio (*S*/*N*) of 3 and 10, respectively. Determined values of *LOD* and *LOQ* for proteins in analyzed solutions were, respectively, 5.4 and 8.4 ng/μL. After recalculation of obtained values on theoretical sample weight of flour (30 mg) and volume used for protein extraction (350 μl) in applied method *LOD* and *LOQ* for proteins in flour were, respectively, 63.5 and 98.5 ppm.

Relative concentrations of the glutenin subunits within the investigated samples, determined using the one-point calibration, which was based on a comparison of the peak area of the protein of interest to the peak area of the upper marker with known protein concentration, are given in Table 2 (sample I-1a) and Table 3 (samples I-1b, I-1c, I-2 and II). Protein concentrations corresponding to the resolved protein bands (Figure 2) were recalculated and expressed in ppm in the flour samples.

Sample I-1a was applied four times on a single Agilent LabChip in order to determine the wheat flour glutenin subunits quantitation reproducibility on the same chip. The mean concentration values ± standard deviations (*SD*) of the resolved proteins signed by their molecular weights are shown in Table 2. Reproducibility of the relative quantitation of the glutenin subunits, analyzed on the same chip and expressed as relative standard deviation (coefficient of variation, *C.V.*) was below 8% (average value for all protein bends in Table 2). Also, HMW-GS quantitation reproducibility was at the same level. Additionally, the sample I-1a was applied on two other Agilent LabChips in order to investigate if individual chip runs affected on the chip-based protein assay quantitation reproducibility (Table 2, column 3). Although, the average *C.V.* value for all protein bands was about 8%, HMW-GS quantitation reproducibility was worse and was 12.9%. However, in intra-chip examinations 10 of 17 glutenin proteins showed higher values of reproducibility of the relative quantitation than in inter-chip examinations. The reason for this might be the fact that samples from different wells of the same chip show slight difference in distinguishing of examined proteins and in this study the samples in inter-chip were always put in the same well position of different chip, whereas in intra-chip they were put in different wells, resulting in slightly different values of the peaks area of examined glutenin subunits and consequently in differences in their quantity. The obtained results confirmed that application of different chips in the analysis of the same sample slightly impaired HMW-GS quantitation reproducibility, which is in agreement with the results of Marchetti-Deschmann *et al.* [15]. The paired *t*-test (paired two sample for means,

Table 2. Glutenin subunits chip-based quantitation analysis results of Arija wheat variety flour sample I-1a (Figure 1); results on a single chip and several chips

Size, kDa	Measurements on a single chip (n = 4)		Measurements on three different chips		Measurements on three chips (n = 6)	
	Mean±*SD*, ppm	*C.V.* / %	Mean±*SD*, ppm	*C.V.* / %	Mean±*SD*, ppm	*C.V.* / %
14.0	89.65±7.44	8.3	92.65±1.85	2.0	90.33±5.88	6.5
15.6	141.8±15.55	11.0	124.9±11.54	9.2	136.3±16.46	12.1
37.5	115.7±4.64	4.0	114.8±5.92	5.2	115.9±4.93	4.3
41.5	271.7±29.77	11.0	262.9±21.90	8.3	272.9±23.32	8.5
44.5	1271±44.2	3.5	1306±62.5	4.8	1293±51.93	4.0
48.7	299.0±29.09	9.7	262.9±9.88	3.8	285.2±31.17	10.9
53.8	157.5±22.79	14.5	175.8±10.57	6.0	161.6±18.75	11.6
59.9	1623±122.2	7.5	1591±74.3	4.7	1621±101.56	6.3
62.1	304.3±26.67	8.8	287.1±12.55	4.4	296.2±24.23	8.2
71.6	261.6±19.73	7.5	247.3±7.50	3.0	257.1±17.43	6.8
97.9	84.12±2.13	2.5	77.10±6.16	8.0	80.71±5.79	7.2
122.7	47.89±2.01	4.2	58.13±9.38	16.1	53.01±8.31	15.7
132.1	138.8±5.21	3.8	112.3±19.74	17.6	126.2±19.97	15.8
140.0	137.5±1.00	0.7	123.6±14.13	11.4	130.6±11.77	9.0
149.5	147.7±16.46	11.1	157.9±12.66	8.0	148.7±13.04	8.8
187.2	346.3±34.20	9.9	375.0±18.80	5.0	352.3±28.04	8.0
219.7	23.33±3.47	14.9	25.79±4.91	19.0	24.97±4.01	16.1
GS, Average *C.V.*		7.8		8.03		9.38
HMW-GS, Average *C.V.*		7.4		12.9		12.2

α = 0.05) that was applied to compare the protein concentrations determined on a single chip and on three different chips, confirmed that there was no statistical difference between the results ($p(T \leq t)$ = 0.45, T = 0.79, t-critical two tailed = 2.12) and ruled out the influence of the chip itself on the protein analysis. Therefore, it can be considered that the application of different chips does not significantly affect HMW-GS quantitation results, that is, the proteins quantitation reproducibility in the real matrix. The mean values (±*SD*) of glutenin subunits' concentrations and the corresponding *C.V.* values, calculated on the bases of six measurements on three chips, are given, respectively, in the last two columns of Table 2. The average *C.V.* value was below 9.5% for all protein bands, and was in a range from 11.4-13.1% for HMW-GS. The highest value of *C.V.* (16.1%) was obtained for the protein band of molecular weight of 220 kDa that was the protein with the lowest determined concentration of 25 ppm. The proteins of the highest concentrations of 1621±102 and 1293±52 ppm in the flour were the LMW-GS compounds that correspond to the protein bands of molecular weights of 59.9 and 44.5 kDa, respectively. The HMW glutenin subunit of the highest concentration of 352.3±28.0 ppm was the one of the molecular weight of 187.2 kDa. The ratio of HMW-GS to LMW-GS was 18%.

In addition to the individual chip influence, there is a possibility that some other steps in the chip-based protein assay, such as the buffer addition steps or extract solution addition steps, might affect the quantitation results.

In order to investigate the influence of the buffer addition step, protein quantitation results of samples I-1b and I-1c, shown in Table 3, were compared to I-1a sample protein quantitation results. Results of paired t-test are given in Table 4. For both pairs, I-1a; I-1b, and I-1a; I-1c, paired t-tests confirmed that there was no statistical difference between HMW-GS concentrations results ($p(T \leq t)$ values were 0.68 and 0.42, respectively), although there were some more substantial differences between the results when all glutenin subunits were concerned ($p(T \leq t)$ values were 0.22 and 0.08, respectively). Quantitation reproducibility for samples I-1b and 1-1c was within ranges determined previously (Table 2).

In order to investigate influence of the extract solution addition step, protein quantitation results of sample I-2, shown in Table 3, were compared to I-1a sample protein quantitation results. Results of the paired t-test, given in Table 4, indicate that there was no statistical difference between two samples' results for HMW-GS concentrations ($p(T \leq t)$ = 0.60). Consequently, this investigation ruled out influence of the extract solution addition and the buffer addition steps of the applied method on HMW-GS quantitative analysis results.

Table 3. Glutenin subunits chip-based quantitation analysis results of Arija wheat variety flour samples I-1b, I-1c, I-2, and II (Figure 1)

Size, kDa	I-1b		I-1c		I-2		II	
	Mean±*SD*, ppm	*C.V.* / %	Mean±*SD*, ppm	*C.V.* / %	Mean±*SD*, ppm	*C.V.* / %	Mean±*SD*, ppm	*C.V.* / %
14.0	119.5±5.62	4.70	106.8±15.82	14.81	357.5±13.33	3.73	165.8±3.43	2.07
15.6	129.1±13.49	10.46	161.1±12.01	7.45	268.3±1.74	0.65	149.3±6.29	4.21
37.5	121.8±2.61	2.15	121.8±3.61	2.96	223.1±5.22	2.34	179.6±2.29	1.27
41.5	252.7±21.63	8.56	283.1±19.12	6.76	621.8±17.96	2.89	391.7±54.33	13.87
44.5	1308±51.5	3.94	1322±52.1	3.94	1424.3±1.2	0.08	1299±144.2	11.10
48.7	287.1±38.94	13.57	307.4±22.83	7.43	477.5±73.01	15.29	171.0±2.86	1.67
53.8	186.6±20.50	10.99	203.4±12.96	6.37	209.8±10.43	4.97	177.9±15.44	8.68
59.9	1659±63.3	3.82	1665±40.1	2.41	1470±33.0	2.25	1886±282.5	14.98
62.1	268.7±24.72	9.20	235.6±17.57	7.46	407.9±61.42	15.06	311.1±83.50	26.84
71.6	273.0±6.57	2.41	283.8±13.78	4.86	191.8±13.33	6.95	231.6±8.58	3.70
97.9	84.03±4.84	5.76	86.59±4.04	4.67	83.01±10.00	12.05	59.48±4.58	7.69
122.7	50.37±4.27	8.47	45.66±4.58	10.03	94.95±28.89	30.43	66.34±4.58	6.90
132.1	143.3±10.10	7.05	124.9±12.17	9.75	146.0±5.86	4.02	151.5±10.90	7.19
140.0	133.9±10.37	7.47	129.5±20.21	15.60	91.0±11.18	12.29	134.5±8.46	6.29
149.5	164.3±8.96	5.46	166.1±10.04	6.05	126.3±9.27	7.34	166.8±11.60	6.95
187.2	333.8±13.76	4.12	357.5±16.58	4.64	316.4±23.18	7.33	376.9±58.91	15.63
219.7	24.11±4.68	19.40	30.56±8.62	28.21	17.38±2.90	16.67	35.46±11.44	32.26
GS, Average *C.V.*		7.52		8.43		8.49		10.08
HMW-GS, Average *C.V.*		8.71		12.38		13.01		12.54

Table 4. t-Pared test results - comparison of the results of sample I-1a to the other samples

Test	I-1b		I-1c		I-2		II	
	T	*p*	*T*	*p*	*T*	*p*	*T*	*p*
GS (t-critical two tailed = 2.119)	-1.270	0.222	-1.902	0.075	-2.077	0.054	-1.594	0.130
HMWGS (t-critical two tailed = 2.571)	-0.347	0.680	-0.888	0.415	0.551	0.605	-4.677	0.005

The multi-step extraction procedure, which had been applied in order to remove albumin, globulin and gliadin proteins prior to glutenin subunits analysis, was compared to one-step extraction procedure removing only gliadin proteins (sample II, shown in Table 3). Results given in Table 4 indicate that there was statistically significant difference between HMW-GS quantitation results of multi-step and one-step extraction procedures applied prior to glutenin subunits extraction step ($p(T \leq t)$ = 0.005, T = -4.68, t-critical two tailed = 2.57). Concentrations of HMW-GS determined in the isolate that has been obtained by one-step extraction procedure (Sample II, in Table 3) were 11.5% higher than the HMW-GS concentrations determined in sample I-1a, obtained by multi-step extraction procedure. However, statistically significant difference was not confirmed when concentrations of all glutenin subunits were concerned. The ratio of HMW-GS to LMW-GS was 19% and that is lower than the ratios of 23 Western-Siberian wheat varieties from study conducted by Baláz *et al.* [20].

CONCLUSION

Multi-step and single step extraction procedures were used to extract glutenins from the Serbian common wheat variety, Arija. Good resolution of the glutenin subunits bands in molecular weight range from 14.0 to 220 kDa was achieved by the chip-based protein analysis system. Six protein bands corresponding to HMW-GS were in molecular weight range from 120 to 220 kDa. The reproducibility of HMW-GS sizing and quantitation were good, with the average coefficient of variation values of 1.2 and 12.2%, respectively. The ratio of HMW-GS to LMW-GS was about 20%. The investigation ruled out influences of the extract solution addition and the buffer addition steps of the applied method, as well as the individual chip influence on GS and HMW-GS quantitation results. However, statistically significant difference between HMW-GS quantitation results of multi-step and one-step extraction procedures applied prior to glutenin subunits extraction step was confirmed. Concentrations of HMW-GS determined in the isolate that had been obtained by one-step extraction procedure were 11.5% higher than the HMW-GS concentrations determined in the sample, obtained by multi-step extraction procedure.

Nomenclature

GS glutenin subunits
HMW-GS high-molecular-weight glutenin subunits
LMW-GS low-molecular-weight glutenin subunits
SDS-PAGE sodium-dodecyl-sulfate polyacrylamide gel electrophoresis
Mean (of n values) $\text{Mean} = \sum_{i=1}^{n} x_i \Big/ n$
SD standard deviation:

$$SD = \sqrt{\sum_{i=1}^{n}(x_i - \text{Mean})^2 \Big/ (n-1)}$$

C.V. coefficient of variation:

$$C.V. = SD \times 100/\text{Mean}$$

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NAUČNI RAD

POUZDANOST *LAB-ON-A-CHIP* METODE U ODREĐIVANJU GLUTENINSKIH PODJEDNICA VELIKIH MOLEKULSKIH MASA

Polimerni proteini endosperma pšenice, a pogotovo podjedinice glutenina velikih molekulskih masa (HMW-GS) su verovatno najzanimljivija proteinska frakcija koja daje osnovne informacije o pecivnom kvalitetu pšeničnog brašna. Relativno nova metoda koja ima veliki potencijal za brzo, pouzdano i automatizovano analiziranje čistoće, veličine i kvantifikacije proteina je mikrofluid ili Lab-on-a-Chip (LoaC) kapilarna elektroforeza. Cilj ovoga rada je bio da ispita mogućnosti primene LoaC metode, za analiziranje uzoraka proteina izolovanih iz hlebne sorte pšenice iz Srbije, sa posebnim osvrtom na korake koji mogu da dovedu u pitanje pouzdanost rezultata i utiču na reproduktibilnost količina dobijenih gluteninskih podjedinica. Postignuta je dobra rezolucija proteinskih bendova u opsegu molekulskih masa od 14,0 do 220,0 kDa. Reproduktibilnost veličina i količina HMW-GS su bile dobre sa prosečnim vrednostima koeficijenta varijacije od 1,2 i 12,2%. Odnos količina HMW-GS prema količinama gluteninskih podjedinica malih molekulskih masa (LMW-GS) je bila oko 20%. Rezultati istaživanja isključuju uticaje koraka dodavanja rastvora za ekstrakciju i koraka dodavanja pufera za kvantifikaciju primenjene metode, kao i individualni uticaj čipa na rezultate kantifikacije GS. Međutim, postoje statistički značajne razlike između rezultata kvantifikacije HMW-GS kada su primenjeni postupci ekstrakcije iz više i jednog koraka koji su prethodili ekstrakciji gluteninskih podjedinica.

Ključne reči: pšenica, podjedinice glutenina, Lab-on-a-Chip, sorta pšenice Arija, LoaC Lab-on-a-Chip.