ISSN 2308-4057. Foods and Raw Materials, 2016, vol. 4, no. 2

FUNCTIONAL TECHNOLOGICAL PROPERTIES AND ELECTROPHORETIC COMPOSITION OF MODIFIED WHEAT GLUTEN

V. V. Kolpakova[a,*], L. V. Chumikina[b], L. I. Arabova[b], D. N. Lukin[a], A. F. Topunov[b], and E. I. Titov[c]

[a] All-Russian Research Institute of Starch Products,
Nekrasova Str. 11, Kraskovo, Moscow region, 140051 Russian Federation

[b] Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences,
Leninsky Ave. 33 bld. 2, Moscow, 119071 Russian Federation

[c] Moscow State University of Food Production,
Volokolamskoje Shosse 11, Moscow, 125080 Russian Federation

* e-mail: val-kolpakova@rambler.ru

Received June 05, 2016; Accepted inrevised form September 18, 2016; Published December 30, 2016

Abstract: This article provides data on correlation between functional technological properties of native and modified wheat gluten and its specific molecular weights, with an objective to develop control methods for adjustment of physical and chemical specifications of protein products. We used methods for chemical composition analysis in protein products, protein electrophoresis (PAGE), and DWG modifications. We used enzymatic preparations (EP) for DWG properties modification: endoprotease EP (Protamex®) and Flavourzyme 500 MG, which contains both endoprotease and endopeptidase simultaneously. It is shown that native DWG underperforms in its functional technological properties in comparison to sodium caseinate, soy flour, soy concentrate, and egg albumin, therefore its properties are modified by limited proteolysis with protein hydrolysis degree of 1.10–3.41%. Our findings indicate that hydrolysis duration might be used to control DWG properties: to increase solubility, foam forming capacity (FFS) up to the respective values demonstrated by egg albumin, and at the same time, to reduce water- and fat-binding capacity and fat emulsifying capacity. DWG with improved FFS contains single-chain polypeptides, both with low molecular weight (ME) (under 40 kDa), and with medium ME (40–60 kDa). Among multi-chain peptides with more pronounced foam-forming capacity, presence of single-chain peptides with low ME (12–16 kDa) seems more preferable than polypeptides with medium (27–39 kDa) and high ME (69–108 kDa). Revealed regularities in correlation of DWG functional properties and ME / composition specificity are intended to be used in DWG modification for further various applications in food industry, mostly for pastries production.

Keywords: dried wheat gluten, modification, proteolysis, electrophoresis, functional technological properties

DOI: 10.21179/2308-4057-2016-2-48-57 *Foods and Raw Materials*, 2016, vol. 4, no. 2, pp. 48–57.

INTRODUCTION

Protein products of plant and animal origin are extensively used in food industry due to an ample array of functional technological properties (FTP) they possess, such as solubility, water and oil binding capacity, capacity to create stable emulsions, foams, etc. [1]. Proper FTP is a prerequisite for optimal quality indicators, as well as for enhancing nutritive and biological value of food products. Dried wheat gluten (DWG) is a byproduct of wheat starch manufacture, and it definitely requires a wider scope of utilisation. Nowadays, DWG is mostly used in flour milling and bread baking industry [2, 3]. First benefit of DWG is its natural origin, as this product is obtained from cereals by washing them with water without any chemicals involved or any demanding process requirements; the second benefit that should be mentioned is its unique technological properties, with no equivalent substitution in bread baking industry. Gluten proteins improve the strength of wheat flour dough and consumer properties of baked products. An addition of some 1–2% of DWG increases flour protein content and water absorption capacity of weak flour dough, enhances bread output, improves its texture and softness, provides an extended shelf life [4, 5]. In bread baking and flour milling industry, DWG might be used without any modification, however, its FTP do not meet the specifications of various processes in pastry production, such as solubility, foaming capacity requirements, etc. [6]. In order to achieve a wider scope of application for DWG in food industry, new efficient methods of FTP modification are being developed that deal with physical, chemical, and structural properties of proteins: surface hydrophobicity, molecular weights [7], etc.

Eduble proteins are modified by physico-chemical, chemical, and enzymatic methods. The first category

Copyright © 2016, Kolpakova et al. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), allowing third parties to copy and redistribute the material in any medium or format and to remix, transform, and build upon the material for any purpose, even commercially, provided the original work is properly cited and states its license. This article is published with open access at http://frm-kemtipp.ru.

comprises protein treatment by heat [8], pressure [9], ultrasound [10], extrusion [11]; the second, protein atecylation [12], succinylation [13], phosphorylation [14], dilute acid treatment [15], the third group of methods includes hydrolysis [16], and deamination [17]. Proteins are also modified when being exposed to combinations of various factors: temperature and enzymes [18], temperature and pressure [19], proteolysis and ultrafiltration [20], among others. Protein modification results in changes in solubility, fat-emulsifying capacity (FEC) and other properties of gluten [6, 7, 16, 18, 21]. Some researchers [6, 7, 21] determined molecular weights (MW) of gluten proteins and its FTP, however, there have been no studies of a correlation between MW of proteolytically modified DWG proteins and various rheological properties and FTP that would aim to controlling the latter through the adjustment of the former. Samples of both DWG and raw native gluten might vary in their rheological properties that are known to be determined by differences in their molecule size [22]. Consequently, their functional properties might also be different. There are no reports in literature on the dependence of functional properties of DWG proteins on MW of its components (single-chain and multiple-chain polypeptides). Therefore, the objective of this study was to evaluate the FTP of DWG in comparison with properties demonstrated by other protein products, and to investigate the correlation of these properties in native and hydrolysed DWG samples that reveal different rheological parameters with MW specificity in various types of polypeptides separated by polyacrylamide gel electrophoresis (PAGE).

OBJECTS AND METHODS OF STUDY

Objects. We used the following samples: two DWG samples produced by BM company (Kazakhstan), a sample of egg albumin (manufacturer: Eurovo S.R.L., Italy), a sample of sodium caseinate (PC Milk, Belarus), a sample of soy isolate Supro 760 (Protein Technology International, US), a sample of defatted soy flour 200/80 (Cargill, Belgium), and a sample of soy concentrate (Technomol, Russia). Quality and safety of the protein products were confirmed by certificates of conformity issued by Rospotrebnadzor (the Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing). All the protein products except soy flour were classified as concentrates and isolates (Table 1) according to their protein content, and different in their carbohydrates, ash, and fat content.

We used enzymatic preparations (EP) for protein modification: endoproteases (Protamex®) and endo- and exopeptidases (Flavourzyme 500 MG) manufactured by Novozymes (Denmark), with proteolytic activity of 125 and 85 E/g, respectively. EP activity was determined by Rukhlyaeva's modification of the classic Anson assay as per GOST 20264.2-88; this method is based on hydrolysis of the sodium caseinate protein by the EP and further sedimentation of the non hydrolyzed protein by trichloroacetic acid.

Chemical composition. Mass fraction of protein in protein products was determined by the Kjeldahl method, in Kjeltek analyzer manufactured by Tecator (Sweden) as per GOST 10846-91; humidity, as per GOST 9404-88; ash, as per GOST 27494-87. Mass fraction of fat was analyzed as per GOST 29033-9, while fiber was determined by Kürschner-Hanak method as exposed in the guidelines [23]. Mass fraction of carbohydrates was calculated by subtracting respective mass fractions of protein, fat, ash, and humidity from a total of 100 g.

Preparation of regenerated DWG and determination of its rheological properties. 4 g (±0.01 g) of DWG were placed in a porcelain cup and added by 8 cm^3 of tap water at 18–20°C. Then a dough ball was formed, covered by glass and left for 20 minutes to rest. Then gluten was washed out onto a seave, with a jet of tap water, for 15–20 minuted at the same temperature. Regenerated 4 g gluten was manually pressed out to drain water, then a required amount of 4 g (±0.01 g) was separated and molded into a smooth-surfaced ball, without discontinuities. The gluten ball was placed to rest into a cup of water at 18–20ºC for 15 minutes. Then the compressive deformation (elasticity) ($N_{def.}$) was measured in the IDK-1 apparatus, as per the test technique provided in GOST 27839-2013.

Table 1. Chemical composition of protein products

Protein products	Humidity, %	Mass fraction (% of dry weight)				
		Protein	Fat	Carbohydrates	Fibre	Ash
Egg albumin	8.0 ± 0.4	87.0 ± 0.3	2.0 ± 0.2	2.0 ± 0.2	3.0 ± 0.5	6.0 ± 0.5
Sodium caseinate	6.0 ± 0.2	88.0 ± 0.6	2.0 ± 0.2	1.0 ± 0.5	4.0 ± 0.4	5.0 ± 0.3
Soy flour	9.0 ± 0.2	43.0 ± 0.4	14.0 ± 0.1	34.0 ± 0.8	5.0 ± 0.6	4.0 ± 0.2
Soy concentrate	4.0 ± 0.5	61.0 ± 0.2	5.0 ± 0.1	26.0 ± 0.7	4.0 ± 0.2	4.0 ± 0.4
Soy isolate	4.0 ± 0.7	92.0 ± 0.5	0.5 ± 0.2	1.5 ± 0.3	3.0 ± 0.3	3.0 ± 0.1
DWG	4.0 ± 0.1	75.0 ± 0.5	1.0 ± 0.4	22.0 ± 0.6	1.0 ± 0.1	1.0 ± 0.2

Hydrolysis degree. Hydrolysis degree of DWG proteins (Dh) was calculated according to the equation

$$Dh = Cs/Cc*100\%,$$

where C_s was total nitrogen of the amino acids within the sample (%) [29], and C_c is the amino nitrogen content in the hydrolyzed sample.

Total amino acid nitrogen was obtained after complete hydrolysis of the sample quantity (approx. 100 mg) with 10 cm^3 6 mol/dm^3 HCl solution. The sample was maintained in a thermostat at 110ºC for 24 hours, then amino nitrogen was determined by formol titration [23]. To determine amino nitrogen, the required sample quantity was mixed with certain amount of distilled water (assuming the moisture content of 63%) with a diluted proteolytic EP. Dough was made and kept in a thermostat for certain time, at a suitable temperature. Then the hydrolysate (4.5 g) was added with 65 cm^3 of distilled water, the mixture was dispersed in a homogenizer for 4–5 minutes, then centrifuged at 6000 rpm for 20 minutes. The supernatant liquid was drained, with 5 cm^3 transferred to a cuvette with distilled water (20 cm^3) for titration. The mixture was neutralized by 0.2 N of NaOH solution under pH meter control. When pH level of the solution reached 7, it was added with 0.5 cm^3 of formaldehyde with phenolphtalein, and the mixture was titrated with 0.2 N NaOH solution up to pH 9.1–9.5, which corresponded to bright red coloring of the sample. Hydrolysed DWG samples were dried in a lyophilic drying plant Continuous Freeze Dryer (US).

Electophoresis and molecular weights. In order to determine MW and polypeptide composition of the proteins, a required quantity (0.2–0.5 g) of DWG and its hydrolysates was mixed with 20 cm^3 of buffer containing 62.5 mM of Tris-HCl, 8 M of urea, 2% SDS, and 0.01% of bromphenol blue (pH 6.8). The dispersion was agitated for 1 hour at room temperature. On the next day, (in 15–17 hours) the samples were agitated once again, for 1 hour at room temperature, then centrifuged at 12 000g for 20 minutes. Protein content in solutions was determined by the Kjeldahl method in BUCHI K-424 analyzer (Switzerland).

In a reductive cleavage reaction with native and hydrolyzed proteins, sample buffer was used, with 5% content of 2-mercaptoethanol. The buffer was added to the precipitate obtained during the previous centrifugation, in the amount of 10 cm^3. The sample was incubated at 37°C for 2 hours, then centrifuged at 12 000g for 20 minutes. The centrifugate was used for the analysis of polypeptide protein composition by a one-dimensional electrophoresis; separating gel contained 10–20% of acrylamide (pH 8.8), while stacking gel, 6% (pH 6.8). Electrode buffer contained Tris-glycine (pH 8.3) and 0.1% SDS. Electrophoresis was performed at 4–6°C in 6–8 hours, with permanent electric current of 25–30 mA. Protein content in the samples was analyzed by the Bramhall [24] method with certain modifications. Standard MW markers (manufactured by Sygma, Germany) were used to calculate protein molecular weights: phosphorylase b (MW 92 kDa), bovine serum albumin (MW 69 kDa), catalase (MW 60 kDa), egg albumin (MW 46 kDa), aldolase (MW 36 kDa), carbonic anhydrase (MW 29 kDa), trypsin inhibitor (MW 20 kDa), cytochrome C (MW 12 kDa).

Functional technological properties. In order to determine solubility of the protein products, a required quantity of 2–4 g was suspended in 30 cm^3 of distilled water for 1 hour in an agitator, then the resulting dispersion was left overnight at 4ºC. Then it was agitated again, for 1 hour, then centrifuged at 16000*g* for 15 minutes, and the resulting centrifugate was drained into a volumetric flask for 100 cm^3. The solution was made up to the mark, protein content was determined by the Kjeldahl method and expressed in percentage of the total protein within the required sample amount.

In order to determine **water-binding capacity** (WBC), 1 g of the protein product was added into 25 cm^3 of distilled water, the mixture was mixed in a homogenizer at 1000 g for 1 minute and centrifuged at 8000*g* for 15 minutes. As the supernatant was drained, the centrifuge tube was turned upside down and placed onto filtering paper. In 10 minutes, the tube was weighed, and the WBC was calculated, expressed in grams of bound water per 1 g of the product. **Fat-binding capacity** (FBC) was determined by weight. 1 g of the product was added into 25 cm^3 of sunflower oil, mixed for 1 minute at 1000 g and centrifuged at 8000 g for 15 minutes. The supernatant was drained, the tube was turned upside down and placed onto filtering paper. In 10 minutes, the tube was weighed, and the FBS was calculated as a ratio of oil weight bound with the product and the original oil weight. The parameter was expressed in grams of bound fat per 1 g of the product. In order to determine fat emulsifying capacity (FAC), a required amount of 3.5 g of a protein product was placed into a mixer, then 50 cm^3 of distilled water were added. The mixture was suspended for 1 minute at 4000 g, then combined with 50 cm^3 of cornflower oil and emulsified for 5 minutes at 8000 g. The emulsion was poured into 4 tubes and centrifuged for 5 minutes at 2000 g. FAC was calculated as a ratio of emulsion volume and overall system volume expressed as a percentage. **Emulsion prepared according to the above procedure was tested for stability** (ES) after 30 minutes heating at 80°C. Then the emulsion was cooled for 15 minutes, distributed into 4 tubes, and centrifuged for 5 minutes at 2000 g. ES was determined as a ratio of emulsion volume and overall system volume expressed as a percentage. In order to determine foam forming capacity (FFC), a required amount of a protein product (0.6 g) was placed in a glass, diluted with 25 cm^3 of distilled water, and the mixture was thoroughly triturated with a glass stick. Then the resulting mass was transferred into a 100 cm^3 cylinder, the rest of the required amount was flushed with water, and total liquid volume was made up to 30 cm^3. The sample was held horizontally and agitated for 1 min, while the foam height was measured. FFC was calculated as a ratio of foam level and original liquid level, expressed as a percentage. **Foam stability** (FS) of protein products was

determined as a ratio of remaining foam level after 15 minutes of static immobility, and original foam level, expressed as a percentage.

Statistical data analysis. All results are presented as average values of 3–5 experiments with dispersion and correlation analysis and the Q-test. A multiple comparison analysis was carried out to assess significant differences among the samples. Fisher's least significant difference (LSD) test was used to describe means with 95% confidence.

RESULTS AND DISCUSSION

Functional technological properties in different types of protein products

FTP analysis in protein products of plant and animal origin, as summarized in Table 2, showed that soy products demonstrated maximum water-binding capacity, while in DWG samples, this property was most limited. The highest FBC was demonstrated by soy isolate and soy concentrate, the lowest, in both samples of wheat gluten. Maximum FAC and FFC were found in egg albumin. In DWG, these properties are more pronounced then in sodium caseinate and in soy products, except FS. At the same time, DWG FTP did not reach the values found in egg albumin, which is most widely used in pastries production as a conventional foam forming, fat emulsifying, and water-binding angent. Therefore, we further carried out a study on modification of DWG FTP intended for application of this protein product not only in bread-baking industry, but also for pastries and other foods production.

DWG modification and determination of its functional technological properties

Functional properties of DWG were modified by limited proteolysis, with proteolytic enzymes applied as per the technique presented in [25] work. Differences in rheological properties of the regenerated DWG samples were taken into account. These properties were determined by organoleptic methods and by IDK-1 apparatus. It was found that DWG sample No. 1 had a low compressive deformation index (40 instr. units), while DWG sample No. 2, on the contrary, had a high index, 80 instr. units. The DWG sample No.1 was characterized as "short-tearing", with stretching above the ruler less than 10 cm, while the DWG sample No. 2 was "weak", stretching for 22 cm. Based on the above, hydrolysis of the DWG sample No.1 was run with endoprotease EP Protamex®, while hydrolysis of the DWG sample No. 2, with Flavozyme 500 MG, which contained both endoproteinase and exopeptidase. At the same time, we assumed the previously stated regularities: a stronger DWG sample positively relates to a more efficient application of endoproteinase, and vice versa, a weaker DWG sample is more likely to be efficiently treated with exopeptidase [25]. In both cases, EP concentration amounted to 0.3 E/g of protein, with pH 6.5 ± 0.2, and temperature 50ºC. DWG hydrolysis time with Protamex® was adjusted from 10 to 80 min., with Flavozyme 500 MG, from 40 to 160 min.

Other researchers [26, 27] have studied properties of modified proteins by using their soluble components, while we investigated the FTP of hydlolyzed DWG proteins jointly, with both soluble and insoluble parts present, assuming that protein behavior in a food system would depend on simultaneous presence of both fractions. FTP of the DWG obtained by different hydrolysis duration was evaluated in accordance with the following parameters: WBC, FBC, FAC, FFC, ES, FS, and solubility. Our findings in functional properties of hydrolysates in comparison with native DWG samples properties are summarized in Table 3. It is seen that native DWG is diluted in water by 3.2–3.6%, while hydrolyzed DWG, by 31.3–35.5%. By the end of hydrolysis, solubility of short-tearing DWG was increased by 3.6–7.2 times, of weak DWG, by 4.8–10.8 times. Maximum solubility was observed in weak gluten proteins after 2–2.5 hours of hydrolysis. Foam-forming properties of hydrolysates were also better than the ones found in native samples. As hydrolysis time was increased up to 85 minutes in DWG No.1 and up to 160 minutes in DWG No. 2, protein FFC was increased by 55 and 82%, respectively, thus exceeding absolute FFC values of eff protein (283%) (Table 2). By the end of hydrolysis process, foam stability of the samples increased by 41–51%. At the same time, hydrolysis degree of proteins in the DWG sample No. 1 amounted to 2.21–2.89%, in the DWG sample No. 2, to 3.22–3.41%.

In contrast to FCC and solubility, such protein properties as FAC, ES, WBC, and FBC were gradually reduced as hydrolysis time was increased. Thus, for example, within the hydrolysis time range of 85–160 minutes, protein FAC was decreased by 6–7 times, WBC, by 4.5–9.0 times. In hydrolyzed short-tearing DWG, FFC, FBC and solubility proved to be somewhat higher than in weak DWG, while both FAC and WBC were lower instead. Thus, we can adjust FTP of DWG by controlling hydrolysis time, as we increase its solubility and foam forming capacity, while reducing all other parameters.

Sodium dodecyl sulfate - polyacrylamide gel electrophoresis

Considering that protein hydrolysis degree reflects changes in molecular weight distribution of polypeptides [16], we further performed electrophoresis of hydrolyzed DWG proteins in comparison to native proteins. For that purpose, we used DWG No. 1 and DWG No. 2 hydrolysates obtained during 80 minutes of hydrolysis, with FFC values of 310% and 243%, respectively. Hydrolysis degree was almost the same in both samples (2.86–2.89%), however, their properties were different. Table 4 and Fig. 1 provide results of polypeptides MW determination by PAGE electrophoresis with no use of mercaptoethanol (columns 1, 2, 5, 6) and with mercaptoethanol (columns 3, 4, 7, 8). Mercaptoethanol is known to reduce disulfide bonds in multi-chain proteins, which produces single-chain polypeptides with free sulfhydryl groups that migrate into gel.

Table 2. Functional technological properties of protein products

Protein products	WBC, g/g	FBC, g/g	FAC, %	ES, %	FFC, %	FS, %
Egg albumin	soluble in water	1.15 ± 0.10	70 ± 2	75 ± 3	283 ± 3	90 ± 2
Sodium caseinate	forms gel	1.64 ± 0.00	57 ± 1	47 ± 1	177 ± 2	60 ± 3
DWG, sample No. 1	1.2 ± 0.3	0.66 ± 0.30	62 ± 3	68 ± 2	200 ± 6	46 ± 0
DWG, sample No. 2	0.9 ± 0.1	0.60 ± 0.40	59 ± 1	65 ± 3	174 ± 2	45 ± 0
Soy flour	1.6 ± 0.2	1.20 ± 0.30	49 ± 1	47 ± 1	80 ± 1	60 ± 1
Soy concentrate	7.4 ± 0.5	2.20 ± 0.20	61 ± 2	48 ± 3	50 ± 3	68 ± 2
Soy isolate	7.9 ± 0.5	1.80 ± 0.60	55 ± 1	55 ± 1	55 ± 4	68 ± 1

Table 3. Functional technological properties of native and hydrolyzed DWG samples with different rheological properties

Indicators	$N_{def.}$ 40 instr. units.					$N_{def.}$ 80 instr. units.				
	Control	Hydrolysis time, minutes				Control	Hydrolysis time, minutes			
		10	40	60	80		40	80	120	160
WBC, g/g	1.20 ± 0.07	0.71 ± 0.07	0.58 ± 0.08	0.42 ± 0.05	0.13 ±0.07	0.90 ± 0.04	0.75 ± 0.70	0.61 ± 0.70	0.32 ± 0.70	0.20 ± 0.70
FBC, g/g	0.66 ± 0.07	0.64 ± 0.06	0.39 ± 0.12	0.44 ± 0.03	0.60 ± 0.11	0.60 ± 0.09	0.45 ± 0.08	0.45 ± 0.13	0.72 ± 0.02	0.43 ± 0.13
FAC, %	62.0 ± 2.0	54.0 ± 1.0	38.0 ± 2.0	14.0 ± 0.0	11.0 ± 1.0	59.0 ± 1.0	54.0 ± 0.5	30.0 ± 0.5	14.0 ± 2.0	8.0 ± 1.5
ES, %	60.0 ± 1.0	50.0 ± 2.0	39.0 ± 1.0	15.0 ± 2.0	10.0 ± 1.0	59.0 ± 0.5	52.0 ± 1.0	31.0 ± 2.0	14.0 ± 2.0	9.0 ± 0.5
FFC, %	200 ± 2	216 ± 1	250 ± 3	300 ± 3	310 ± 2	174 ± 2	216 ± 1	243 ± 3	276 ± 4	316 ± 3
FS, %	46.0 ± 1	50.0 ± 0.5	56.0 ± 0.5	60.0 ± 1.0	65.0 ± 1.0	45.0 ± 0.5	48.0 ± 1.0	57.0 ± 2.0	63.0 ± 2.0	68.0 ± 1.0
Solubility, %	3.6 ± 0.2	13.1 ± 0.4	16.2 ± 0.5	26.2 ± 0.4	31.3 ± 0.5	3.2 ± 0.5	15.3 ± 0.4	27.2 ± 0.4	35.1 ± 0.1	35.5 ± 0.2
Proteolysis degree, %	0	1.10 ± 0.05	1.72 ± 0.07	2.21 ± 0.11	2.89 ± 0.06	0	1.76 ± 0.06	2.86 ± 0.03	3.22 ± 0.08	3.41 ± 0.05

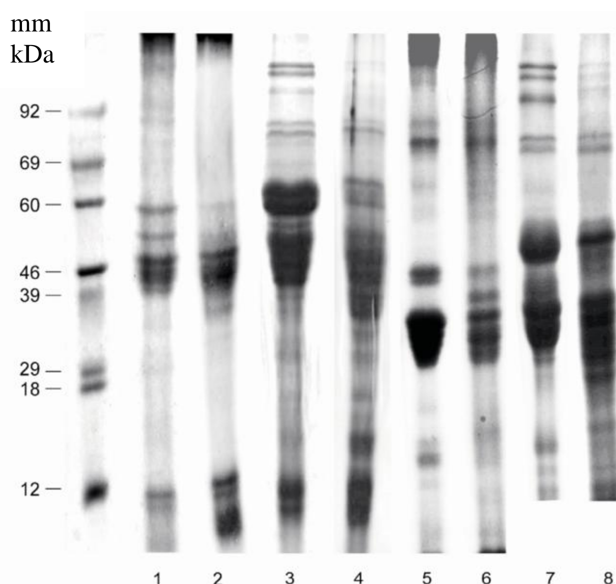

Fig. 1. Polypeptide composition of dry wheat gluten of different quality: Strips 1, 2, 3, 4 – short-tearing gluten; Samples 5, 6, 7, 8 – weak gluten; 1, 3, 5, 7 – native gluten; 2, 4, 6, 8 – hydrolyzed gluten; 1, 2, 5, 6 – without mercaptoethanol; 3, 4, 7, 8 – with mercaptoethanol.

Comparison of single-chain proteins MW distribution in native DWG samples (columns 1 and 5) shows that short-tearing gluten, which has a 26% higher FFC than weak gluten, lacks two low molecular polypeptides, with MW from 14 to 24 kDa. Comparison of hydrolysates composition (columns 2, 6) reveals that HDWG No. 1, with a 67% higher FFC than HDWG No. 2 (Table 2) lacks polypeptides with ME of 20, 22, 36, 39 kDa, but has proteins with ME MM 14, 54 and 60 kDa. What both HDWG samples (columns 2, 6) have in common is a reduced baseline amount of proteins with MW 120 kDa, dissociation of high molecular peptides (MW 108–110 kDa), medium molecular (MW 69, 86 kDa), and one low molecular peptide (MW 24 kDa). At the same time, HDWG sample demonstrated FFC 69–110% higher than native samples (columns 1, 5). HDWG sample No. 1 (short-tearing gluten) which had the highest FFC (column 2), even in comparison to HDWG No. 2 (weak gluten) contained no polypeptides with MW 36 and 39 kDa. Therefore, a common characteristic found in gluten protein composition in various quality samples is presence of a single-chain peptides group with MW from 40 to 60 kDa, and a peptides group with MW from 12 to 16 kDa, as well as absence of peptides with MW from 69 to 110 kDa. This characteristic ensures high FFC. Peptides with ME of 20–24 and 36–39 kDa also did not facilitate FFC increase up to 310 % of egg albumin value.

Considering that during the electrophoresis experiment a significant part of proteins remained at the baseline, we further analyzed samples of native DWG and its hydrolysates with mercaptoethanol that breaks disulfide bonds and ensures polypeptide penetration into a gel. We discovered that across the spectrum of native and hydrolyzed DWG samples of different quality with mercaptoethanol, at the baseline there were no proteins with MW > 120 kDa (columns 3, 4, 7, 8), which indicated total dissociation of all disulfide bonds. On the other hand, after disulfide bonds dissociation, a native sample of short-tearing DWG demonstrated new polypeptides with MW of 22–30 kDa and with MW of 84–110 kDa (column 3), while a weak DWG sample revealed proteins with MW from 18 to 33 and from 54 to 108 kDa (column 7).

A particularity of composition found in the DWG sample No.1 (column 4), as compared to the native DWG sample, was trace quantities of polypeptides with MW 92, 108, 110 present in multi-chain proteins, and complete absence of two polypeptides with MW 28–36, and four, with MW from 69 to 100 kDa. In weak DWG hydrolysate (column 8), multi-chain proteins contained the same high molecular peptides (MW 86–110 kDa), as the native sample did (column 7), however, peptides with medium and low MW were also present. An exception was made for peptides with MW 24 and 60 kDa. Therefore, native DWG and its hydrolysates contained both single-chain and multi-chain polypeptides, which corresponds to the data on content and structure of native raw gluten proteins [22].

Composition analysis of multi-chain proteins in HDWG No. 1 hydrolysate (column 4) with the highest FFC observed (310%), revealed that its distinctive feature, as in comparison to HDWG No. 2 hydrolysate (column 8), was presence of trace quantities of high molecular peptides (MW 92–110 kDa), and complete absence of six peptides with MW from18 through 39, of four peptides with MW from 69 through 102, and proteins with MW over 120 kDa. Judging by the strips intensity, this sample contained less polypeptides with medium MW (40–60 kDa) and more polypeptides with the lowest MW (12–16 kDa).

As we discuss our findings, we should note that in food industry, egg products (eggs, melange, egg powder) are conventionally used as a source of surfactants to create protein foam and emulsion systems; however, these egg product also contain fat and cholesterol. Therefore, there is an ongoing search

for alternative sources in order to reduce calories of final products and at the same time, to exclude egg albumin that some people are allergic to [28]. Along with this purpose, plant-based and animal proteins obtained from both conventional and unconventional feedstock are gradually getting more applications [14]. Just like proteins found in other cereals (rye, oats, rice, corn), gluten is poor in essential amino acids (lysine, threonine, tryptophan), however, due to its unique rheological properties it is widely used in bread baking [29]. Interacting with each other through ionic, hydrogen, disulfide, and other bonds, polypeptide chains of gliadin and glutenin form a tridimensional structure with hydrophobic and hydrophilic groups located onto its surface. Properties of this structure determine elasticity, stretching, cohesion, softness, and eventually, volume of bread, elasticity and porosity of its crumb [22]. Our findings suggest that the structure of unmodified gluten proteins does not ensure prominent functional properties for them to be used in complex pastries systems, as a foam forming or emulsifying agent (structure former) (Table 2). DWG FTPs might be achieved by controlling its proteins with proper physico-chemical, chemical, and enzymatic methods. However, due to their intrinsic safety, biotechnological methods based on use of various proteases [16], including endoproteases and exopeptidases, seem to have considerable advantages over other methods. Endoproteases hydrolyze peptide bonds from inside, as they dissociate protein molecules into smaller fragments, while exopeptidases proceed to sequential dissociation of the ends of amino acids, one by one (Fig. 2).

We used endoproteinsase EP Protamex® for proteolysis of an elastic DWG sample, as we had previously [25] established that with this EP, hydrolysis degree in time under 100 minutes is higher that with Neutrase®, which conforms with findings reported by other authors who speak of its higher efficiency [16]. However, other researchers have not considered a correlation of functional properties with proteins MW in DWG demonstrating different rheological properties. According to our data, hydrolysis of short-tearing DWG with Protamex® and of weak DWG with Flavourzym 500 MG, leads to a formation of peptide mixture with higher solubility, FFC and FS, in comparison with native gluten. This regularity is reverse to the one established for hydrolysis of rice middlings proteins with papain and alcalase [30], but it conforms with findings of the research [7] which demonstrated that improved solubility and FFC at the same protein hydrolysis degree (2.8–2.89%) might be achieved through fractioning of DWG hydrolysates by ultrafiltration. The regularity we established for WBC and FAC reduction in DWG proteins during hydrolysis do not conform with the data obtained by those authors. Therefore, properties of DWG hydrolysates are unique for each case, they depend on the rheological properties of gluten and on the enzyme type, and thus require specific case studies, which is confirmed by the findings of the present work. During protein proteolysis, new absorbed active peptides appear, consequently, additional suface activity arises, which indicates a possibility to improve protein quality and to obtain valuable nutrition ingredients. Each protein requires specific modification studies with relation to various functional properties.

According to our findings, protein hydrolysis and FTP improvement (hydrolysate FFC achieved FFC of egg albumin, about 300%) were accompanied by formation of single-chain peptides, not only with very low (below 15 kDa) [7] and low MW (below 40 kDa), as it had previously been shown [16], but also with medium MW (40–60) kDa (Table 4) – values that correspond to ω-gliadin MW, partially to γ-gliadin and low molecular glutenin subunits [31]. This is equally confirmed by comparison of our findings with data obtained in [32], where the authors showed that at 180% FFC of DWG hydrolysates with papain (which is lower than the value we obtained, 300%) polypeptides with low MW (5–15 kDa) are typical, therefore, we might conclude that polypeptides with MW of 40–60 kDa participate in ensuring enhanced foam forming capacity and solubility, along with low molecular peptides.

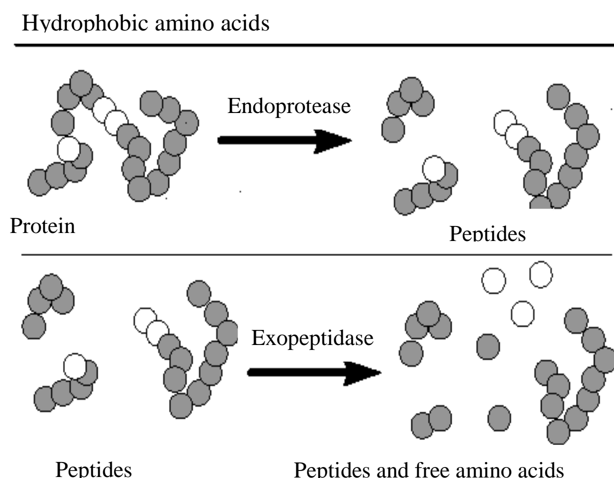

Fig. 2. Endoproteases and exoproteases action on proteins.

Table 4. Molecular weights of polypeptides present in DWG of different quality, kDa

Short-tearing gluten (DWG No. 1)				Weak gluten (DWG No. 2)			
DWG	HDWG	DWG	HDWG	DWG	HDWG	DWG	HDWG
without mercaptoethanol		with mercaptoethanol		without mercaptoethanol		with mercaptoethanol	
1	2	3	4	5	6	7	8
> 120	> 120↓	–	–	> 120	> 120↓	–	–
–	–	110	110 tr	110	–	110	110↓
108	–	108	108 tr	–	–	108	108
–	–	-	–	–	–	102	102 tr
–	–	100	–	–	–	100	100 tr
92	–	92	92 tr	92	–	92	92 tr
86	–	86	–	86	–	86	86 tr
–	–	84	–	–	–	–	–
69	–	69	–	69	–	69	69
60	60	60	60↓	60	–	60	–
54	54	54	54↓	–	–	54	54
46	46	46	46↓	46	46	46	46
44	44	44	44↓	44	44	44	44 tr
40	40	40	40↓	40	40	40	40
39	–	–	–	39	39	39	39
36	–	36	–	36	36	36	36
–	–	–	–	–	–	33	33
–	–	30	30	–	–	30	30
–	–	28	–	–	–	28	28 tr
–	–	–	–	–	–	27	27
–	–	–	–	24	–	24	–
–	–	22	22	22	22	22	22
–	–	–	–	20	20	20	20
–	–	–	–	–	–	18	18
16	16	16	16↑	16	16	–	–
14	14	14	14↑	–	–	–	–
12	12↑	12	12↑	12	12↑	–	–

Note. *DWG – control sample; HDWG – hydrolyzed sample; “tr” – trace quantities of strips, ↑ – increased intensity of strips; ↓ – decreased intensity of strips.

We have discovered a relationship between FTPs and composition not only in single-chain polypeptides present in DWG of different quality, but in multi-chain polypeptides as well. If the electrophoretic spectrum of proteins had remained the same during our experiments with mercaptoethanol, just as it had been without it, that would have meant that the proteins were composed of a single polypetide chain. However, as the proteins were composed of polypeptide chains connected with disulfide bonds, incubation with mercaptoethanol would lead to their dissociation, and separate polypertides migrated into a gel, thus changing the spectrum outlook. Multi-chain proteins of both DWG hydrolysates with higher FFC and solubility than in native samples demonstrated total absence or trace quantities of peptides with MW from 69 to over

120 kDa (Table 4, columns 4, 8). However, they contained larger amounts of polypeptides with MW from 12 to 20 kDa, that is, low molecular components in multi-chain proteins are equally important for ensuring the properties under consideration. This conclusion is confirmed by the fact that multi-chain proteins of the HDWG sample No. 2 (column 8), with lower FFC (243%) and solubility (27.2%) values in comparison to HDWG No. 1 (FFC 310%, solubility 31.3%) (column 4), contained polypeptides with high MW (69–108 kDa) and medium MW (27–39 kDa), while low molecular peptides (MW 12–16 kDa) were not present. The same regularity might be observed while comparing the composition of multi-chain proteins in native DWG of different quality (column 3, 7). Short-tearing DWG with solubility of 3.6%, and FFC, 200% as compared to weak DWG with solubility of 3.2% and FFC, 174%, lacked two polypeptides with MW 84–102 kDa and four peptides with MW from 24 to 39 kDa, however, it contained low molecular peptides with MW 12–16 kDa. The above described specificity of MW in polypeptides with higher FFC, FS, and solubility, were also correlated with lower WBC and FAC values. Relationship between FBC and ME of polypeptides was not observed in this work.

Thus, a comparative study of native DWG FTPs in contrast to other protein products revealed the utility of improving these for future application in foam systems for pastries production, considering its specific rheological properties. Depth of proteolysis for weak DWG that would improve solubility, FFC, and FS, should be achieved with hydrolysis degree of 1.76 through 3.41%, under exposure to endoproteinase and exopeptidases. As for short tearing DWG, hydrolysis degree should amount to 1.1 through 2.89%, run with endoproteinase. DWG with higher FFC, FS, and solubility contained single-chain polypeptides not only with low MW (below 40 kDa), but with medium MW as well (40–60 kDa). The highest values of FFC, FS, and solubility were found in short-tearing gluten, with 80 minutes proteolysis time leading to formation of single-chain polypeptides with MW 54 and 60 kDa, and elimination of peptides with MW from 20 through 39 kDa. We revealed a dependence of FTP on MW characteristics for both single-chain and multi-chain polypeptides determined by PAGE electrophoresis run with and without mercaptoethanol. A distinctive feature of multi-chain polypeptides united by covalent disulfide bonds within DWG is presence of proteins with low MW (12–16 kDa), which are more favorable for enhancement of foam forming capacity and solubility than polypeptides with medium (27–39 kDa) and high MW (69–108 kDa**).** Multi-chain peptides found in short-tearing gluten with the highest FFC level (310%) lacked 4 polypeptides with MW from 69 to 102 kDa, and a group of peptides with MW from 27 to 33 kDa, however, they contained low molecular peptides (MW 12–16 kDa). Use of modified DWG with well researched MW characteristics of single-chain and multi-chain protein components, with hydrolysis degree of 1.10–3.41% and higher solubility that native DWG, seems promising as a foam forming agent for pastries products containing foams and foam-emulsions.

ACKNOWLEDGEMENTS

This work was supported by grants from the President of the Russian Federation "Scientific school 5834.2014. 4".

REFERENCES

1. Lee H.C., Htoon A.K., Uthayakumaran S., and Paterson J.L. Chemical and functional quality of protein isolated from alkaline extraction of Australian lentil cultivars: Matilda and Digger. *Food Chemistry*, 2007, no. 102, pp. 1199–1207. DOI: 10.1016/j.food.chem.2006.07.008.
2. Dulaev V., Melechkina E., Anisimov A., et al. Enrichment of wheat bread flour with dry gluten at flour milling facilities. *Bread products*, 2004, no. 10, pp. 32–34. (In Russian).
3. Kolpakova V., Yudina T., Severinenko S., and Vanin S. Dry wheat gluten – efficient flour improving agent. *Bread products*, 2006, no. 10, pp. 50–53. (In Russian).
4. Lazaridou A., Duta D., Papageorgiou M., et al. Effects of hydrocolloids on dough rheology and bread quality parameters in gluten free formulations. *Journal of Food Engineering,* 2007, vol. 79, no. 3. pp. 1033–1047. DOI: 10.1016/*j*.jfoodeng.2006.03.032.
5. Noorfarahzilah M., Lee J.S., Sharifudin M.S., et al. Applications of composite flour in development of food products. *International Food Research Journal,* 2014, vol. 21, no. 6, pp. 2061–2074.
6. Wang J., Zhao M., Yang X., and Jiang Y. Improvement on functional properties of wheat gluten by enzymatic hydrolysis and ultrafiltration. *Journal of Cereal Science,* 2006, vol. 44, no. 1. pp. 93–100. DOI: 10.1016/ 1.ics.2006.04.002.
7. Saberi A.H., Kadivar M., and Keramat J. Improvement of functional properties dlutens extrated from two Iranian wheat varieties (Sardari and Mahdavi) employing chemical and enzymatic modifications. *Journal of Agricultural Science and Technology,* 2008, vol. 56. pp. 243–252.
8. Zhang H., Claver I.P., Li Q., et al. Structural modification of wheat gluten by dry heat-enhanced enzymatic hydrolysis. *Food Technology and Biotechnology*, 2012, vol. 50, no. 1. pp. 53–58.
9. Ahmed J., Ramaswamy H.S, Ayad A., et al. Effect of high-pressure treatment on rheological, thermal and structural changes in Basmati rice flour slurry. *Journal of Cereal Science,* 2007, vol. 46, no. 2. pp. 148–156. DOI: 10.1016/ j.jcs.2007.01.006.
10. Zhang H., Claver I.P., Zhu K.-X., and Zhou H. The effect of ultrasound on the functional properties of wheat gluten. *Molecules,* 2011, no. 16, pp. 4231–4240. DOI: 10.3390/molecules1605423.

11. Surówka K., Zmudzinski D., and Surówka J. Enzymic modification of extruded soy protein concentrates as a method of obtaining new functional food components. *Trends in Food Science and Technology,* 2004, vol. 15, no. 1–2, pp. 153–160. DOI: http://dx.doi.org/10.1016/j.tifs.2003.09.013.
12. Majzoobi M. and Abedi E. Effects of pH changes on functional properties of native and acetylated wheat gluten. *Journal homepage*, 2014, vol. 21, no. 3, pp. 1219–1224.
13. Krause J.-P., Mothes R., Schwenke K.D. Properties of interfacial films formed by succinylated legymin from faba beans (*Vicia faba L.*). *Colloids and Surfaces B: biointerfaces*, 1997, vol. 8, no. 6, pp. 279–286. DOI: http://dx.doi.org/10.1016/S0927-7765(96)01330-6.
14. Braudo E.E. (ed.). *Rastitel'nyy belok: novye perspektivy* [Plant protein: new perspectives]. Moscow: Pishheprom-izdat Publ., 2000, 180 p.
15. Lee J.-Y., Lee H.D., and Lee C.-H. Characterization of hydrolysates produced by mild-acid treatment and enzymatic hydrolysis of defatted soybean flour. *Food Research International*, 2001, vol. 34, no. 2–3, pp. 217–222. DOI: http://dx.doi.org/10.1016/S0963-9969(00)00155-1.
16. Kong X., Zhou H., and Qian H. Enzymatic hydrolysis of wheat gluten by proteases and properties of the resulting hydrolysates. *Food Chemistry,* 2007, vol. 102, pp. 759–763. DOI: 10.1016/j.foodchem.2006.06.062.
17. Webb M.F., Naeem H.A., Schmidt K. Food Protein Functionality in a Liquid Sistem: A comparison of deamidated wheat protein with dairy and soy proteins. *Journal of Food Science,* 2002, V. 67. pp. 2896–2902.
18. Wang J.S., Wei Z.Y., Li L., et al. Characteristics of enzymatic hydrolysis of thermal-treated wheat gluten. *Journal of Cereal Science,* 2009, vol. 50, no. 2, pp. 205–209. DOI: 10.1016/j.jcs.2009.05.004.
19. Kieffer R., Schurer F., Kohler P., and Wieser H. Effect of hydrostatic pressure and temperature on chemical and functional properties of wheat gluten: Studies on gluten, gliadin and glutenin. *Journal of Cereal Science,* 2007, vol. 45, no.3, pp. 285–292. DOI: 10.1016/j.jcs.2006.09.008.
20. Popineau Y., Huchet B., Lare B., and Berot S. Foaming and emulsifying properties of fractions of gluten peptides obtained by limited enzymatic hydrolysis and ultrafiltration. *Journal of Cereal Science,* 2002, vol. 35, no. 3. pp. 327–335. DOI: 10.1006/jcrs.2001.0437.
21. Rachman A.T.M.M., Sana N.K., Khan Md.M.H., et al. Partial purification deamidase from germinating wheat (*Triticum Aestivum L*) seeds and its application for the improvement of functional properties of wheat gluten. *Journal of Bioscience,* 2007, vol. 15, pp. 89–98. DOI: 10.3329/jbs.v15i0.2208.
22. Kolpakova V.V. Molecular aspects of rheological properties of gluten, dough, quality of bread. *Applied biochemistry and microbiology*, 1994, vol. 30, no. 5–6, pp. 535–549. (In Russian).
23. Nechaev A.P. (ed.), Traubenberg S.E., Kochetkova A.A., et al. *Pishchevaya khimiya: Laboratornyy praktikum* [Food chemistry: Laboratory practice manual]. St. Petersburg: GIORD Publ., 2006, 133 p.
24. Bramhall S., Noack N., Wu M., and Loewenberg J.R. A simple colorimetric method for determination of protein. *Analytical Biochemistry,* 1969, vol. 31, no. 1, pp. 146–148. DOI: http://dx.doi.org/10.1016/0003-2697(69)90251-6.
25. Kolpakova V.V., Chumikina L.V., Vasil'ev A.V., et al. Wheat gluten proteolysis by enzyme preparations of directional action. *International Journal of Agronomy and Agricultural Research*, 2014, vol. 5, no. 2. pp. 72–86.
26. Linares E., Larre C., and Popineau Y. Freeze- or spray-dried gluten hydrolysates: Biochemical and emulsifying properties as a function of drying process. *Journal of Food Engineering,* 2001, vol. 48, no. 2, pp. 127–135. DOI: 10.1016/S0260-8774(00)00148-5.
27. Bean S. and Tilley M. Separation of water-soluble proteins from cereals by high-performance capillary electrophoresis (HPCE). *Cereal Chemistry,* 2003, vol. 80, no. 5, pp. 505–510. DOI: 10.1094/chem.2003.80.5.505.
28. Opinion of the scientific panel on dietetic products, nutrition and allergies on a request from the commission related to a notification from WFA and the AWRI on albumin (egg white) used in the manufacture of wine pursuant to Article 6, paragraph 11 of Directive 2000/13/EC – for permanent exemption from labeling. *The EFSA Journal,* 2007, no. 566, pp. 1–7. DOI:10.2903/j.efsa.2007.566.
29. Kaushik R., Sharma N., Swami N., et al. Physico-chemical properties, extraction and characterization of gluten from different Indian wheat cultivars. *Research & Reviews: A Journal of Crop Science and Technology,* 2013, vol. 2, no. 1, pp. 1–6.
30. Hou L., Zhu Y., and Li Q. Characterization and preparation of broken rice proteins modified by proteases. *Food Technology and Biotechnology,* 2010, vol. 48, no. 1, pp. 50–55.
31. Shewry P.R. and Tatham A.S. Biotechnology of wheat quality. *Journal of the Science of Food and Agriculture*, 1997, vol. 73, no. 4, pp. 397–406.
32. Wang J.S., Zhao M.M., Bao Y., et al. Preparation and characterization of modified wheat gluten by enzymatic hydrolysis-ultrafiltration. *Journal of Food Biochemistry,* 2008, vol. 32, no. 3, pp. 316–333. DOI: 10.1111/j.1745-4514.2008.00157.

Please cite this article in press as: Kolpakova V.V., Chumikina L.V., Arabova L.I., Lukin D.N., Topunov A.F., and Titov E.I. Functional technological properties and electrophoretic composition of modified wheat gluten. *Foods and Raw Materials*, 2016, vol. 4, no. 2, pp. 48–57. DOI: 10.21179/2308-4057-2016-2-48-57.