Protein profiles of buckwheat, rye and oat during in vitro gastro-duodenal digestion

ZDENKA GÁLOVÁ – EVA PALENČÁROVÁ – ANDREA ŠPALEKOVÁ – DANA RAJNINCOVÁ – JANKA DRÁBEKOVÁ

Summary
The aim of this study was to analyse three different plant species: buckwheat (cv. Pyra), rye (cv. Oklon) and oat (cv. Valentin) and to study profiles of raw flour proteins and changes throughout simulated in vitro gastro-duodenal digestion. The kinetics of used digestions was studied as performed by various incubations between 0 min to 120 min for simulated gastric digestion and, for combined digestion 120 min for gastric digestion, followed by 0 min to 180 min for simulated duodenal digestion. The digestion process was monitored by using sodium dodecyl sulfate polyacrylamide gel electrophoresis. Proteine profiles of raw flour were different from those at the end of simulated in vitro digestion. The majority of polypeptides disappeared after complete gastro-duodenal digestion. Some of them (55 kDa, 31 kDa, 36 kDa, 45 kDa) were found to be completely or partly resistant to hydrolysis at certain stages of digestion.

Keywords
buckwheat; rye; oat; in vitro digestion

The human body obtains the energy and nutrients from food and our cells absorb these nutrients after digestion, which is a complex process of breaking down food molecules into energy and other useful components. Then they can be absorbed into the bloodstream and distributed throughout the body to maintain good health [1]. In the human body, digestion takes place in the gastrointestinal tract, which has four main functions: 1. to ingest the eaten food, 2. breaks the food down into simple chemical components for energy and nutritional purposes, 3. extract nutrients (e.g. macronutrients such as carbohydrates, fats or proteins, as well as micronutrients like vitamins and minerals, and 4. expel waste products and unusable products of digestion [2]. A number of important digestive hormones and digestive enzymes help to regulate digestion, especially in the upper gastrointestinal tract and important progress has been made in understanding the development of this topic over the last two decades [3].

Protein digestion is considered in terms of the function of different parts of the gastrointestinal tract. Proteins from the diet are mechanically broken down in the oral cavity by the action of chewing, also known as mastication. During this, the food is mixed with saliva and forms a bolus [4]. Saliva lubricates the bolus, which eases its transportation to the stomach via the oesophagus. Saliva has an early, limited role in total digestion by beginning the breakdown of starch with amylase, a major component of parotid saliva, that initially dissolves sugar. Salivary enzymes also initiate fat digestion [5]. This results in the release of proteins associated with starch, making it more available to proteolytic enzymes in the gut. Both oral and gastric phases are important steps in digestion, because they induce the first, often extensive, chemical and physical transformation of food, and

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they control the transit of solid and liquid phases of gastric content [6]. However, protein digestion begins in the stomach, where pepsin hydrolyses proteins into oligopeptides of widely varying sizes. Fluctuations in gastric emptying times, gastric pH, pepsin activity levels and the extent of emulsification result in variable and often incomplete hydrolysis of proteins. Proteins are further processed in the duodenum, the first segment of the small intestine. When gastric contents enter the small intestine, pH rises rapidly to near neutrality due to pancreatic and duodenal bicarbonate secretion. At the same time, under the influence of the hormone cholecystokinin, bile and pancreatic enzymes are secreted into the duodenum. There are five recognized pancreatic proteolytic enzymes, namely, trypsin, chymotrypsin, elastase, carboxypeptidase A, carboxypeptidase B. The pancreatic enzymes digest incoming proteins and polypeptides into short peptides (typically 2–6 residues in length), which are further processed by brush border oligopeptidases and ultimately absorbed as amino acids, dipeptides and tripeptides through the intestinal lining into capillaries in the villi [7–9].

However, besides providing nutrients and energy, food can be also a vehicle of allergy. Our knowledge about the causes of food allergy is limited. Scientists suspect that certain genes, as well as conditions in the stomach and intestine, may play a role in development of food allergy. If the food is not broken down properly in the intestine, it may be that food allergens are made available for the intestine immune system in a way that promotes their allergenicity. Therefore, individuals with reduced stomach acidity may be predisposed to becoming food allergic or it may worsen the symptoms of a pre-existing food allergy. Infants may be more susceptible to developing food allergy because they have an immature intestine [10, 11].

A way to study the function of the intestine in connection to normal and also pathological function is the use of in vitro digestion models. Several in vitro digestion systems have been developed. These systems proved to be a useful tool for understanding the dynamic digestion of various food components held within the structure of a food matrix. The proteins and starch digestion from cereal food depends on both the extent and the kinetics at which starch and proteins are hydrolysed. However, the rate of digestion is the mainly considered parameter [12].

In the present study we applied in vitro gastro-duodenal digestion of storage proteins from buckwheat (cv. Pyra), rye (cv. Oklon) and oat (cv. Valentin) with an intention to better understand hydrolysis of these proteins. The changes in protein profiles throughout simulated in vitro digestion were clarified in detail.

MATERIALS AND METHODS

Three plant species were used in this analysis, namely, buckwheat (cv. Pyra), rye (cv. Oklon) and oat (cv. Valentin). Their grains were obtained from the Gene Bank of the Research Institute of Plant Production (Piešťany, Slovakia) and were milled by CU Mill (Lionhill Company, London, United Kingdom) to a homogenous flour with particle size of approximately 0.2 mm. In vitro gastro-duodenal digestion of raw flour was carried out according to the method of MANDALARI et al. [13, 14] at the Institute of Food Research in Norwich, Great Britain. Chemicals and enzymes, which were used within in vitro gastro-duodenal digestion of raw flour, were obtained from several companies: enzyme co-lipase (EC 3.1.1.3) from porcine pancreas was from Roche (Basel, Switzerland), egg L-phosphatidylcholine from Lipid Products (Surrey, United Kingdom) and enzymes pepsin (EC 3.4.23.1), α-chymotrypsin (EC 3.4.21.1), porcine trypsin (EC 3.4.21.4), porcine pancreatic lipase (EC 3.1.1.3), pancreatic amylase (EC 3.2.1.1), bile salts (Na-taurochlorate and Na-glycolodeoxycholate) and all other chemicals were from Sigma Aldrich (St. Louis, Missouri, USA), unless otherwise stated.

Samples preparation

Proteins were extracted from the flour by adding 1 ml of a buffer to 50 mg of flour. The buffer contained 28 $mg \cdot ml^{-1}$ dithiothreitol (DTT), immobilized pH gradient (IPG) buffer, ultra-pure water and rehydration buffer (urea, thiourea, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate (CHAPS)) for immobilized pH gradient. The samples were then wheel-mixed for 1 h and centrifuged for 3 min at 9 000 ×*g*. Concentration of proteins in the supernatant was determined by Coomassie Plus protein assay (Thermo Fisher Scientific, Waltham, Massachussets, USA), which was based on the Bradford assay [15]. The concentration of proteins of the oat extract was very low, so Compact-Able Protein Assay Preparation Reagent Set (Thermo Fisher Scientific) was used to precipitate the proteins, which were then resuspended in the extraction buffer as described previously. Phospholipid vesicles and hepatic mixture were then prepared. Triplicate samples were set up from gastric and

duodenal digestion. Content of proteins in whole sample was determined by Kjeldahl method and fractional composition of protein complex according to MICHALÍK [16].

Phospholipid vesicles preparation

Phospholipid vesicles were prepared using a modification of a procedure described previously [13, 14]. Solvent was evaporated from a 188 μl aliquot of phospolipid stock solution (63.5 mmol·l^{-1} in chloroform), which was placed into a 50 ml round-bottom flask and dried in a rotary evaporator to make a thin film of phospholipids on the inside wall of the flask. After three purges with nitrogen, the residual solvent was removed under vacuum. Lecithin was suspended in 24.35 ml of the simulated gastric fluid (0.15 mol·l^{-1} sodium chloride, pH 2.5) with approximately five 2 mm diameter glass balls in a flask, which was then placed in a shaking incubator (37 °C, 2.83 Hz). After approximately 30 min, the suspension was sonicated using a titanium-tipped sonication probe (Branson 450 Digital Sonifier, Danbury, Connecticut, USA) at 5 °C in a vessel with a cooling jacket. This was done three times for 90 s with a pulse cycle of 40% full power for 1.5 s on and for 1 s off. After sonication, the suspension was filtered through a nylon syringe filter (pore size 0.22 μm; Nalge Nunc, Rochester, New York, USA) to remove any titanium deposited by the sonicator.

Hepatic mixture preparation

Solvent was evaporated from both 0.768 ml of the lecithin stock solution (63.5 mmol·l^{-1} in chloroform) and from 2.25 ml of 10 mmol·l^{-1} cholesterol stock solution, in a 100 ml round-bottomed flask. The materials were dried to a thin film of phospholipids by rotary evaporator. After three purges with nitrogen, the residual solvent was removed under vacuum. Then, the hepatic mixture was suspended in 7.5 ml of the simulated duodenal fluid (0.15 mol·l^{-1} sodium chloride, pH 6.5) and bile salts, specifically, 67 mg Na-taurochlorate and 50 mg Na-glycolodeoxycholate were added and the solution was placed in an orbital shaking incubator (37 °C, 2.83 Hz) until it was used in the experiment. If the solution was not clear, it was again sonicated and filtered as specified above.

In vitro gastric digestion

Raw flour was split into 12 portions in 5 ml Bijoux bottles, each containing approximately 20 mg of proteins. Then, the flour was suspended in 600 μl of simulated salivary fluid (0.15 mol·l^{-1} sodium chloride, 3 mmol·l^{-1} urea, pH 6.9) and 10 μl of salivary amylase solution (0.12 U·μl^{-1}). Control sample was prepared in the absence of proteases. Samples were mixed for 2 min in an orbital shaking incubator (2.83 Hz) at 37 °C and, afterwards, were suspended in 1.1 ml simulated gastric fluid. The pH was again adjusted to 2.5 with HCl. Then, a volume of 550 μl of phospholipid vesicles in simulated gastric fluid was added to give total liquid volume of 1960 μl. Finally, 100 μl of pepsin solution (pepsin : protein ratio was 1 : 20) was added and samples were placed in an orbital shaking incubator (2.83 Hz) for 10 min at 37 °C. The reaction was stopped at 0, 1, 2, 5, 10, 30, 60, 90, 120 min time points by adding 250 μl of 0.5 mol·l^{-1} ammonium bicarbonate, when increasing pH of the sample inactivated pepsin.

In vitro duodenal digestion

After 2 h of gastric digestion, the pH value of all samples was adjusted to pH 7.0 using sodium hydroxide, followed by the addition of 60 μl of 0.5 mol·l^{-1} bis-tris(hydroxymethyl)aminomethane (Bis-Tris, pH 6.5) and 340 μl of hepatic mixture. The pH value was re-adjusted to pH 6.5 using sodium hydroxide and the samples were placed in an orbital shaking incubator for 10 min at 2.83 Hz to equilibrate to digestion temperature (37 °C). The volumes of 3.98 μl pancreatic lipase (25 U·μl^{-1}), 12.7 μl pancreatic amylase (4 U·μl^{-1}), 16 μl co-lipase (0.5 mg·ml^{-1} simulated duodenal fluid), 5 μl trypsin (0.01 g·ml^{-1} simulated duodenal fluid) and 20 μl chymotrypsin (0.01 g·ml^{-1} simulated duodenal fluid) were added. Proteolysis was stopped at individual time points by addition of 153 μl Bowmann-Birk trypsin-chymotrypsin inhibitor from soybean (0.01499 g·ml^{-1} simulated duodenal fluid) to inhibit trypsin and chymotrypsin in the digestion mixture. Control digestion was performed in the absence of proteases.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed using 10% polyacrylamide NuPAGE Novex Bis-Tris precast gels (Invitrogen, Carlsbad, California, USA). Molecular weight standard (Mark12 Unstained Standard, Invitrogen) was used to determine molecular weight. Gels were fixed in 40% (v/v) methanol containing 100 g·l^{-1} trichloroacetic acid (TCA) and then stained with SYPRO Ruby Stain (Invitrogen) in the dark. After destaining with 10% (v/v) methanol with 60 g·l^{-1} TCA, gels were imaged using a high-resolution molecular imager (Pharos FX Plus; BioRad Laboratories, Hercules, California, USA). Image

analysis of SDS-PAGE gels was carried out using TotalLab 120 (Nonlinear Dynamics, Newcastle, United Kingdom) using automatic process supplemented with occasional manual adjustments. The output of this process was a list of bands defined either by their molecular weight, together with an estimate of the volume under the Gaussian curve corresponding to the band. This curve demonstrates the kinetics of increase or decrease of protein bands´ molecular weight.

RESULTS AND DISCUSSION

In this study, in vitro gastro-duodenal digestion of raw flour led to peptic hydrolysis of proteins. Raw flour proteins entering the in vitro gastro-duodenal digestion system showed a different profile from those at the end of simulated in vitro digestion. The aggregated proteins were hydrolysed and solubilized during the digestion.

Content of proteins and representation of protein fractions

Nutritive and technological quality of cereals and pseudocereals is appreciated by content of proteins in the grain. Based on BOJŇANSKÁ et al. [17] and GÁLOVÁ et al. [18], the low nutritive value of plant proteins is related to the high proportion of proteins fractions like prolamins, which have a low content of essential amino acids (lysine, methionine, arginine). The human organism is unable to synthesize them, so they have to be a part of the food intake. On the other hand, prolamins have high share of unessential amino acids (glutamic acid, proline). Content of proteins is one of the important qualitative markers that influence exploitation of cereals and pseudocereals.

In the analysed materials, content of proteins and their fractions was determined (Tab. 1). The protein content of the grains and the proportion of the individual protein fractions varied probably depending on the agro-ecological conditions of cultivation, the genetic background and during the ripening of the grain [17, 18]. The highest content of proteins was in rye (11.0 %) and the highest nutritive value had buckwheat (45.0 % of albumins and globulins).

Tab. 1. Representation of proteins and their fractions in the grain of buckwheat, rye and oat.

	Buckwheat	Rye	Oat
Proteins [%]	10.1	11.0	8.7
Albumins and globulins [%]	45.0	33.2	26.4
Prolamins [%]	3.0	38.5	15.1
Glutelins [%]	15.0	17.8	45.3

Buckwheat proteins

Gastric and duodenal digestion of buckwheat (Fig. 1, Fig. 2) pointed to rapid degradation of high molecular weight (MW) proteins. High MW bands with MW of 116–200 kDa (Fig. 1) underwent proteolysis immediately after addition of pepsin at the beginning of gastric digestion.

Area from 66 kDa to 97 kDa (Fig. 1, section A) highlighted a polypeptide, which was resistant to digestion under the influence of gastric phase conditions during 120 min and to conditions of the duodenal phase of digestion during 180 min (Fig. 2, section A). The band with MW of 55 kDa increased in amount from 0 min of the gastric phase (Fig. 1) and was resistant to trypsin and chymotrypsin digestion during the next 180 min of the duodenal phase (Fig. 2).

Also, a polypeptide with MW of 36 kDa highlighted increased in amount during the gastric phase (Fig. 1, section B) but, after 120 min of digestion with the enzymes of the duodenum, its degradation occured (Fig. 2, section B). Two other polypeptides with MW of approximately 45 kDa and 31 kDa, respectively, (Fig. 1, section B) showed resistance to the gastric phase of digestion during 120 min, while during the duodenal phase, their partial proteolysis occured but the remaining polypeptides were still present after 180 min (Fig. 2, section B).

The polypeptide with MW of 22 kDa was resistant during the gastro-duodenal digestion (section C in Fig. 1, 2). Another polypeptide, with MW of 13 kDa, was also shown to be resistant to digestive enzymes of the gastro-duodenal digestion (section D in Fig. 1, 2). Likewise, the other resistant polypeptides were highlighted in the area around 3.5–6 kDa (Fig. 1, section E). These results are in agreement with GUO et al. [19], who reported that remaining proteins of albumins, globulins, prolamins and glutelins shared some similarities and they also exhibited a minor band at 20 kDa and a broad band at 10–14 kDa.

In the samples of buckwheat Pyra, we found proteins that were the most resistant to digestion in comparison with other samples, which agrees with the results of GUO and YAO [20] and AHMED et al. [21]. These studies claimed that, compared to isolated proteins of other cereal and pseudocereals, buckwheat flour fractions had relatively lower digestibility. The lower digestibility of these fractions might be also affected by the structural properties of the proteins.

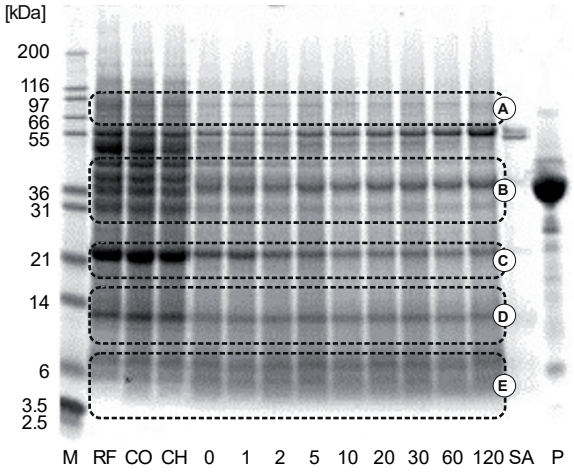

Fig. 1. Protein profile of gastric digestion of buckwheat Pyra.

M – molecular weight marker; RF – raw flour; CO – control (without enzymes); CH – chew material (with all enzymes); 0, 1, 2, 5, 10, 20, 30, 60, 120 – time points (activity of enzymes was stopped by inhibitor in these time points, in minutes), SA – salivary amylase, P – pepsin.
Molecular weight of polypeptides: A – 66–97 kDa, B – 30–50 kDa, C – 22 kDa, D – 13 kDa, E – 3.5–6 kDa.

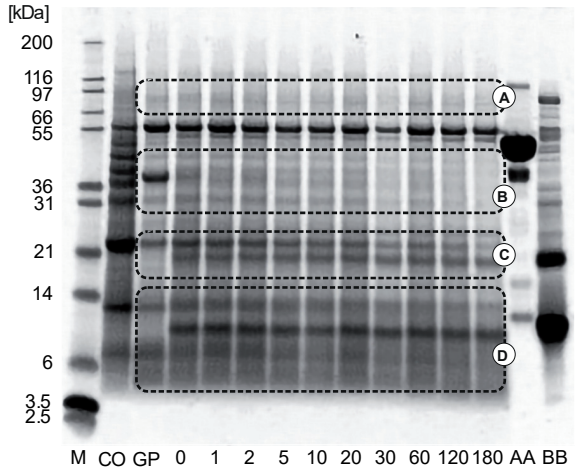

Fig. 2. Protein profile of duodenal digestion of buckwheat Pyra.

M – molecular weight marker; CO – control (without enzymes); GP – gastric point after 120 min of digestion; 0, 1, 2, 5, 10, 20, 30, 60, 120, 180 – time points (activity of enzymes was stopped by inhibitor in these time points, in minutes), AA – alpha amylase, BB – Bowman-Birk trypsin-chymotrypsin inhibitor.
Molecular weight of polypeptides: A – 66–97 kDa, B – 30–50 kDa, C – 22 kDa, D – 13 kDa.

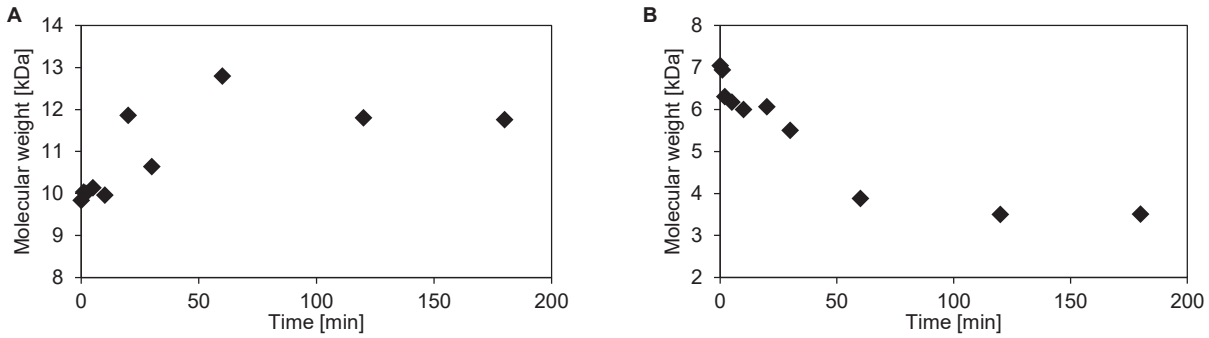

Fig. 3. Effect of in vitro duodenal digestion on buckwheat proteins.

A – the protein band with molecular weight of 55.400 kDa, B – the protein band with molecular weight of 12.826 kDa.

The increase in the 55 kDa polypeptide and the decrease in the 13 kDa polypeptide are shown in Fig. 3.

Rye proteins

In the in vitro digestion profile of whole rye proteins, a polypeptide with MW of approximately 36 kDa was found to be present (Fig. 4, Fig. 5), its MW corresponding to pepsin. After addition of pepsin, the 31 kDa polypeptide underwent proteolysis, weak bands being visible after 120 min of gastric digestion (Fig. 4, Fig. 5). Two polypeptides with molecular weights between 20 kDa and 30 kDa (Fig. 4, section B) were resistant to pepsin digestion and they were also visible after the addition of enzymes of the duodenal phase until the end of the duodenal phase (Fig. 5, section B). The amount of polypeptide with MW of approximately 12 kDa was continuously decreased and the polypeptide was completely hydrolysed after 60 min of pepsin digestion. A resistant polypeptide with MW of approximately 7 kDa was also observed (section C in Fig. 4, 5). A group of polypeptides resistant to trypsin and chymotrypsin digestion during the second stage of digestion for 180 min was observed, having MW of 45–115 kDa (Fig. 5, section A).

The high MW subunits of the proteins of rye were subject to rapid decomposition (Fig. 4, section A). The polypeptide with MW of 66 kDa underwent partial hydrolysis upon pepsin cleavage, but a residuum was still visible after 120 min of the

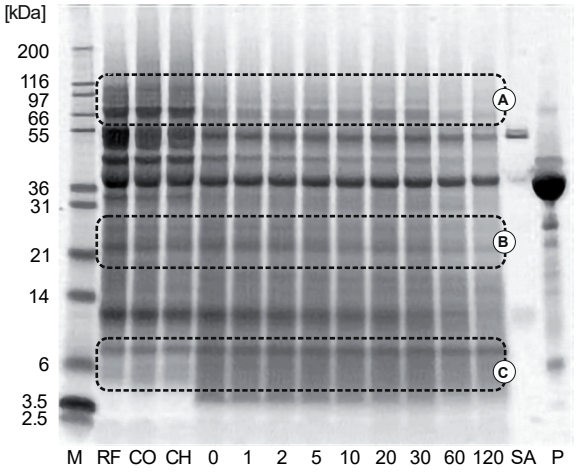

Fig. 4. Protein profile of gastric digestion of rye Oklon.

M – molecular weight marker; RF – raw flour; CO – control (without enzymes); CH – chew material (with all enzymes); 0, 1, 2, 5, 10, 20, 30, 60, 120 – time points (activity of enzymes was stopped by inhibitor in these time points, in minutes); SA – salivary amylase; P – pepsin.
Molecular weight of polypeptides: A – 60–120 kDa, B – 20–30 kDa, C – 7 kDa.

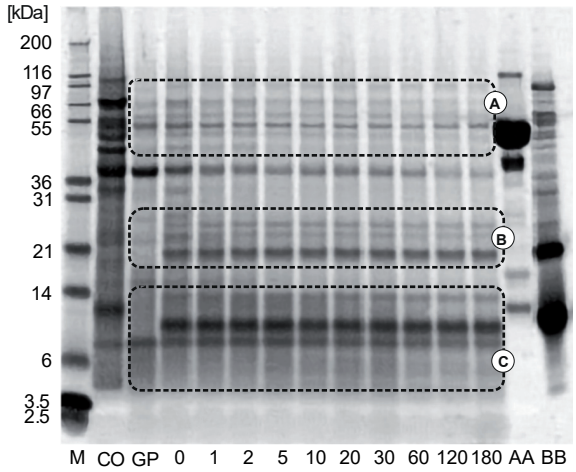

Fig. 5. Protein profile of duodenal digestion of rye Oklon.

M – molecular weight marker; CO – control (without enzymes); GP – gastric point after 120 min of digestion, 0, 1, 2, 5, 10, 20, 30, 60, 120, 180 – time points (activity of enzymes was stopped by inhibitor in these time points, in minutes); AA – alpha amylase, BB – Bowman-Birk trypsin - chymotrypsin inhibitor.
Molecular weight of polypeptides: A – 45–115 kDa, B – 20–30 kDa, C – 7 kDa.

gastric phase. Complete decomposition occured after further 120 min, i.e. after digestion with the enzymes of the duodenal phase. A polypeptide with MW of approximately 55 kDa was resistant to hydrolysis throughout the whole gastro-duodenal digestion (Fig. 4). Intensity of the band corresponding to MW of approximately 45 kDa decreased after addition of pepsin at 0 min (Fig. 4) but the residual polypeptide was again visible after the completion of both of digestion stages (Fig. 5).

The gastric digestion of rye Oklon polypeptides led to rapid degradation of most of them. After gastric digestion of proteins in whole sample, a peptide with MW of 69.4 kDa resisted the gastric and then also the duodenal digestion, the kinetics is shown in Fig. 6. This phenomenon was comparable to resistance of proteins of wheat and barley. By continuing the digestion with duodenal enzymes (simulating the in vivo situation), most of the peptides remaining after gastric digestion were then broken down. Effect of in vitro gastric digestion on 69 kDa polypeptide is shown in Fig. 6.

Solution of partial decomposition of gluten proteins in rye is provided from WALTER et al. [22]. The gluten proteins of rye contribute little to the baking performance of rye flour, which is primarily based on swelling of arabinoxylans at pH 4–5. Therefore, gluten degradation in rye flour is not expected to have a highly detrimental effect on its baking quality. Treatment with a sourdough

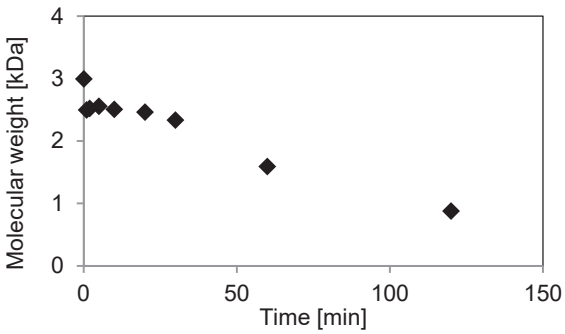

Fig. 6. Effect of in vitro gastric digestion on rye protein with molecular weight 69.419 kDa.

starter and the use of roll-dried sourdoughs, in combination with the use of proteolytic enzymes, such as Flavourzyme (Novozymes, Copenhagen, Denmark), may lead to a significant reduction of the celiac toxicity of prolamins and to preparation of low-gluten bread [23–25].

Oat proteins

The in vitro oat protein digestion profile (Fig. 7, Fig. 8) contains bands in a narrow range of molecular weights (5–66 kDa) compared to the other analysed samples. The polypeptides highlighted on the gastric phase gel with MW of 55 kDa and 66 kDa were resistant throughout the whole gastro-duodenal digestion. In contrast, the polypeptide with MW lower than 55 kDa under-

went proteolysis and was no longer present on the gel after 10 min from the addition of pepsin (Fig. 7). A band of 45 kDa was present on the gel throughout the gastric digestion phase (Fig. 7) but was completely degraded after 60 min from the onset of the duodenal phase (Fig. 8). Again, a resistant polypeptide with MW of 36 kDa, whose molecular weight corresponded to pepsin, was present (Fig. 7). A polypeptide with MW of approximately 31 kDa was hydrolysed after 30–60 min from the addition of pepsin (Fig. 7). Intensities of two protein bands with MW of approximately 25 kDa and 22 kDa also continuously decreased during the gastric phase (Fig. 7), residual polypeptides being visible after 120 min of gastric phase, but they were resistant to enzymes of the duodenal digestion phase (Fig. 8, section B).

Polypeptides with MW of 14 kDa and 7 kDa (section A in Fig. 7 and section C in Fig. 8) were resistant over the entire period of simulated gastro-duodenal digestion. A set of polypeptides that were trypsin-resistant and chymotrypsin-resistant over 180 min in the duodenal phase was observed in a region with MW of 55–100 kDa, 15–25 kDa and 5–14 kDa (Fig. 8, section A, B, C). After 3 h of duodenal digestion, some of the starting proteins remained and they were progressively hydrolysed and converted into peptides with low molecular weight. In the analysis of oat proteins, we noticed an interesting broad band with MW of approximately 36 kDa, intensity of which increased. On the figures of duodenal digestion, this band was less visible, being probably partly digested by duodenal enzymes. This band most likely belonged to pepsin and we observed a similar phenomenon in buckwheat samples in this experiment. Corresponding results were achieved by Comino et al. [26]. They identified oat peptides involved in celiac immune response. MW of all oat protein fractions ranged from lower than 20 kDa to 80 kDa. Polymorphism of the avenin fraction was higher than that of the globulin fraction. MW of oat avenins was from 20 kDa to 36 kDa, with weaker bands of 50 kDa to 70 kDa. Oat glutelin fraction had MW in the range from lower than 20 kDa to 50 kDa. According to Guan et al. [27], who monitored hydrolysis with trypsin, several new bands with MW from 29 kDa to 33 kDa and 14 kDa to 20 kDa appeared after hydrolysis, and the band with MW of 22 kDa remained without a change.

Results of this study correlate with the results of other authors, who successfully applied in vitro digestion models to study structural changes, hydrolysis and digestibility of various protein fractions in food [28–31]. The results obtained by in vitro digestion models are often different from those obtained using in vivo models because of the difficulties in accurately simulating the highly complex physico-chemical and physiological events occurring in animal and human digestive

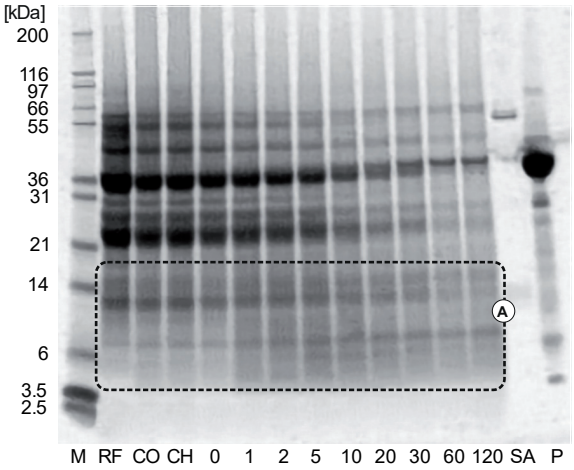

Fig.7. Protein profile of gastric digestion of oat Valentin.

M – molecular weight marker; RF – raw flour; CO – control (without enzymes); CH – chew material (with all enzymes); 0, 1, 2, 5, 10, 20, 30, 60, 120 – time points (activity of enzymes was stopped by inhibitor in these time points, in minutes); SA – salivary amylase, P – pepsin.
Molecular weight of polypeptides: A – 5–14 kDa.

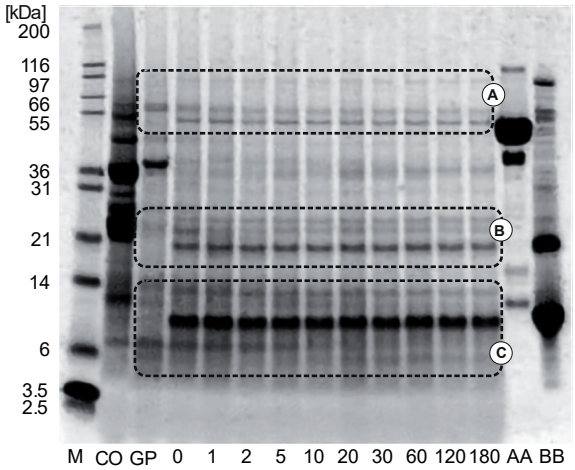

Fig. 8. Protein profile of duodenal digestion of oat Valentin.

M – molecular weight marker; CO – control (without enzymes); GP – gastric point after 120 min of digestion, 0, 1, 2, 5, 10, 20, 30, 60, 120, 180 – time points (activity of enzymes was stopped by inhibitor in these time points, in minutes); AA – alpha amylase; BB – Bowman-Birk trypsin - chymotrypsin inhibitor.
Molecular weight of polypeptides: A – 55–100 kDa, B – 15–25 kDa, C – 5–14 kDa.

tracts. Ideally, food digestion should be studied in vivo in humans but this is not always ethically and financially possible. However, in vitro gastrointestinal digestion systems are a valuable tool for understanding the behaviour of food and food components during digestion. In this study, the most of polypeptides during incubation with enzymes underwent gradual digestion with only traces of the parent proteins remaining after gastric digestion, whose majority disappeared after complete gastroduodenal digestion. Some of proteins were found to be totally resistant to hydrolysis throughout the simulated gastric digestion and some of them also throughout the simulated duodenal digestion.

CONCLUSIONS

From the results of in vitro digestion study of buckwheat, rye and oat storage proteins it can be concluded that the broadest profile and most resistant proteins contained buckwheat, and the narrowest profile was observed in oat. High molecular weight proteins of buckwheat and rye were shown to be easily digestible. In all samples, resistance of the protein of 55 kDa was observed and, in some cases, its amount even increased. Certain polypeptides of analogous resistance in the gastric phase were also observed in buckwheat (MW of 31 kDa and 45 kDa) and oat. A band corresponding to pepsin (36 kDa) was found in all samples. Our findings provide new information on proteins metabolism and confirm that gluten is difficult to degrade. Results demonstrate that the detection of proteins highly depends on the applied methods, thus, the diagnostic tool must be carefully chosen.

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