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REGULAR ARTICLE

IN VITRO GASTRO – INTESTINAL DIGESTION OF WHEAT COELIAC ACTIVE PROTEINS

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ABSTRACT

In vitro gastro-duodenal digestion of wheat coeliac active proteins was examined. Three sets of samples of wheat variety Brea were prepared from gastric and another three from duodenal digested samples. Some of proteins were found to be completely resistant to hydrolysis throughout the simulated gastric digestion and some of them throughout the simulated duodenal digestion. The most of polypeptides during subsequent incubation with enzymes resulted in gradual digestion, with only a trace of the parent proteins remaining after gastric digestion, whose majority disappeared after complete gastro-duodenal digestion. Despite the fact that the most of proteins were broken down by gastro-duodenal digestion into low molecular weight peptides, these molecules retain an allergenic potential.

Keywords: gastro-intestinal digestion, coeliac disease, gluten, SDS-PAGE

INTRODUCTION

The gluten fraction of wheat flour is the main cause of the development of coeliac disease. Determination of individual gluten peptides responsible for toxicity and

immunogenecity is very difficult because, gluten is a mixture of proteins that are closely related (**Wieser and Koehler, 2008)**. The toxicity of α-gliadin was subsequently confirmed by *in vitro* organ culture tests. Later, both *in vivo* and *in vitro* studies implicated all gliadin fractions, with decreasing activity from α- to ω- gliadins, as instrumental in causing disease **(Parnell and Ciclictira, 1999).** A number of important digestive hormones and digestive enzymes help to regulate digestion, especially in the upper gastrointestinal tract **(Montgomery *et al.*, 1999)** and important progress has been made in understanding the development of the human gastrointestinal tract over the last two decades **(Rutsgi and Podolsky, 1997).**

The aim of our work was to study *in vitro* gastro-duodenal digestion of coeliac active proteins in wheat.

MATERIAL AND METHODS

Plant material

Cultivar Brea of wheat *Triticum aestivum* L. was obtained from the Gene Bank of the Research Institute of Plant Production, Piešťany in the Slovak Republic and were milled by CU Mill, (Lionhill Company) to a homogenous flour. Cultivar Brea is the most growing wheat in Slovakia.

In vitro gastric digestion

Raw flour was split into 12 portions in 5 ml Bijoux bottles, each containing approximately 20 mg protein; the protein content was determined by Kjeldahl method. The flour was suspended in 600 µl of simulated salivary fluid (SSF) (0.15 M sodium chloride, 3 mM urea, pH 6.9) and 10 µl of salivary amylase solution (0.12 $U.\mu l^{-1}$) and a control sample was prepared in the absence of proteases. Samples were mixed for 2 min in an orbital shaking incubator (37 °C, 170 rpm) and afterwards were suspended in 1.1 ml simulated gastric fluid (SGF) and pH was readjusted to pH 2.5 with HCl as required. Then, 550 µl of egg L-R-phosphatidylcholine (PC) vesicles in SGF was added to give total liquid volume of 1960 µl. Finally, 100 µl of pepsin solution (pepsin: protein ratio/1:20) was added and samples were placed in an orbital shaking incubator for 10 min at 37^{o}C. The reaction was stopped at 0, 1, 2, 5, 10, 30, 60, 90, 120 min time points by adding 250 µl (0.5 M) ammonium bicarbonate,

increasing pH of the sample to inactivate the pepsin. Samples were stored on ice until required.

In vitro duodenal digestion

After 2 h gastric digestion the pH of all samples was adjusted to pH 7.0 using sodium hydroxide, followed by the addition of 60 µl 0.5 M Bis-Tris (pH 6.5) and 340 µl of hepatic mix. The pH was readjusted to pH 6.5 using sodium hydroxide and the samples were placed in an orbital shaking incubator for 10 min to equilibrate to digestion temperature (37^{o}C). The amounts 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 - SDF), 5 µl trypsin (0.01 g.ml^{-1} SDF), and 20 µl chymotrypsin (0.01 g.ml^{-1} SDF) were added. Proteolysis was stopped at the different time points by addition of 153 µl Bowmann-Birk trypsin-chymotrypsin inhibitor from soybean (0.01499 g.ml^{-1} SDF) above that calculated to inhibit trypsin and chymotrypsin in the digestion mix. Control digestion was performed in the absence of proteases.

In vitro Gastro-Duodenal Digestion of Raw Flour

In this study, experimental digestive treatment time of proteins was comparable to the mean passage time of food through the stomach. To study the kinetics of these digestions, different incubations between 0 s to 2 h for simulated gastric digestion and, for combined digestion 2 h for gastric digestion, followed by 0 s to 3 h for simulated duodenal digestion, were performed. Raw flour with adding no enzymes was used as a control.

Three sets of samples for wheat were prepared from gastric and another three from duodenal digested samples. Total proteins in whole sample, soluble protein in upper aqueous phase, and insoluble proteins in the pellet were followed by both, gastric and duodenal digestion. The digestion process was monitored using SDS-PAGE gels. Image analysis of SDS-PAGE gels was carried out using TotalLab 120 (Nonlinear Dynamics, Newcastle, UK) using an automated process supplemented with occasional manual adjustments.

RESULTS AND DISCUSSION

In vitro gastro-duodenal digestion of wheat proteins helps to understand the types of transformations during digestion and could be used for studying proteins digestion in extreme

or pathological conditions **(Masson *et al.*, 1989).** The process of gastro-duodenal digestion also may play a role in determining the allergenic properties of food proteins. Although the mechanisms by which food allergens sensitize an individual remain currently unclear, most of them are thought to sensitize via the gastrointestinal tract (GIT) and, for this reason, digestibility and gut permeability are key factors to consider since they may affect the allergenic potential **(Aalberse, 2000).** Stability against digestion is consider an important requirement of allergens and eliciting allergic reaction *via* the gastrointestinal route **(Mittag *et al.*, 2004).**

Our results showed, that during gastric digestion of wheat cv. BREA soluble protein in upper aqueous phase, HMW proteins were rapidly degraded with a loss of the polypeptides with Mr ranging between 40-116000 Da that were accompanied by the appearance of a series of fragments of Mr 6-30000 Da together with the very low molecular weight material running at the gel dye front appearing after 1min (Figure 1).

The bands were also rapidly digested, with only a broad band about Mr 12000 remaining after 5 min digestion, while at the same time lower molecular weight polypeptides were formed (4-6000 Da) which remained stable for 2 h gastric digestion, around the time taken for gastric digestion *in vivo*. The similar results obtained **Mandalari *et al.*, (2008)**.

One of the HMW proteins with Mr 97000 remains resistant to 2 h of gastric digestion, but it disappeared at the beginning of duodenal digestion. Polypeptide with Mr 45300 Da was found during gastric digestion, followed by duodenal digestion of whole sample. It was rapidly digested in first 2 min, and then its digestion was slowed-down, but after 3 h of duodenal digestion band was still visible.

On the other hand, peptide with Mr 11200 Da was resistant to 2 h of gastric digestion, but during duodenal digestion was impossible to analyzed it because of high intensity of band with the same molecular weigh that probably belong to Bowman–Birk Trypsin chymotrypsin inhibitor (Figure 2). In our study, we found more similar bands that intensity was higher in comparison with followed bands. As well, the lots of band that belong to used enzymes were found by all plant species. For example, polypeptide with Mr 36000 probably belongs to pepsin (Figure 2).

The gastric digestion started after the 1 min. of action of digestive enzymes, colour intensity of many bands has been decreasing. For example, by gastric digestion of soluble protein, proteins of approximate molecular weight 66300 Da, 14500 Da, 10700 Da, 8800 Da were digested on smaller peptides, intensity of their band decreased during digestion. We can find here interesting band of Mrs about 27300 Da, which appeared in 1 min. of gastric

digestion, subsequently intensity of its band decreased and on gel of duodenal digestion it is not visible, so it was probably digested.

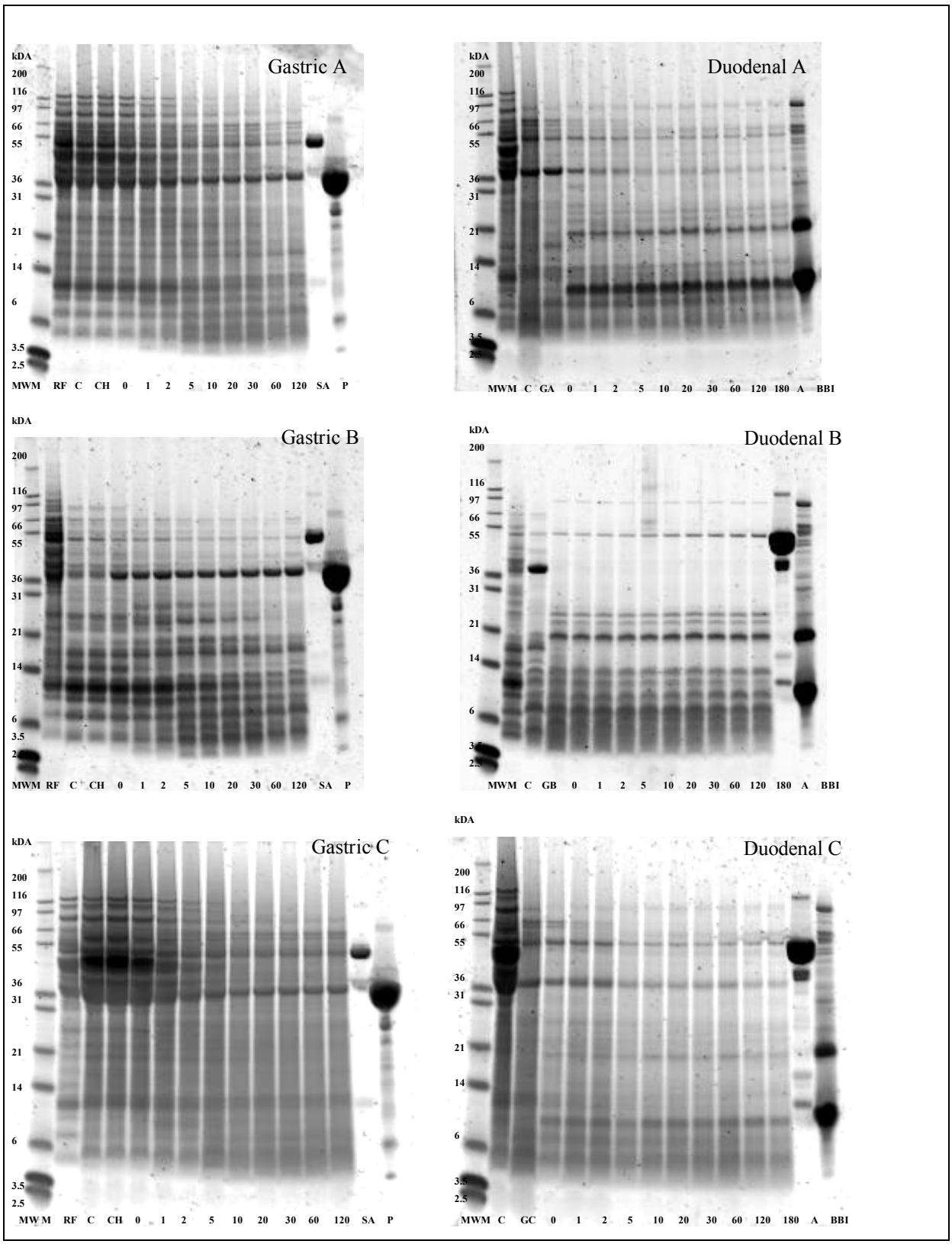

Figure 1 Duodenal and gastric digestion of wheat BREA

Gastric A –gastric digestion of total proteins, Gastric B – gastric digestion of soluble proteins, Gastric C – gastric digestion of insoluble proteins, Duodenal A – duodenal digestion of total proteins, Duodenal B – duodenal digestion of soluble proteins, Duodenal C – duodenal digestion of insoluble proteins. MWM – molecular weight marker; RF – raw flour; C – control (without enzymes); CH – chew material (with all enzymes); 0, 1, 2, 5, 10, 20, 30, 60, 120, 180 – time points (activity of enzymes was stopped by inhibitor in these time points).; SA – salivary amylase; P – pepsin; A – alpha amylase, BBI – Bowman - Birk trypsin - chymotrypsin inhibitor; GA, GB, GC – gastric point after 120 min.

As well as polypeptide with Mrs about 20100 Da, intensity of its band increased until 10 min. of digestion and then intensity of band is decreasing. During duodenal digestion is not properly visible because of stronger intensity band of Bowman–Birk Trypsin chymotrypsin inhibitor (Figure 2).

Polypeptide with Mrs 4800 Da started not to be digested until 30 min of gastric digestion, but on the gel of duodenal digestion is not visible. We can see as well as protein that remained stable for 2 h gastric digestion. For example here belongs peptides with Mrs about 17300 Da, 6600 Da. We can see peptide with Mrs about 13000 Da that appeared in 5 min. of gastric digestion and then it remains stable, but o the gel of duodenal digestion it is not visible.

On the gel of gastric digestion of pellet, band of polypeptides are not so good visible, but we can notice two bands of approximately molecular weight 60000 Da, these stay undigested after 2 hours of digestion, but they are digested after 2 min of duodenal digestion. Again we can see here protein with Mrs about 36000 Da that correspond to pepsin (Figure 2).

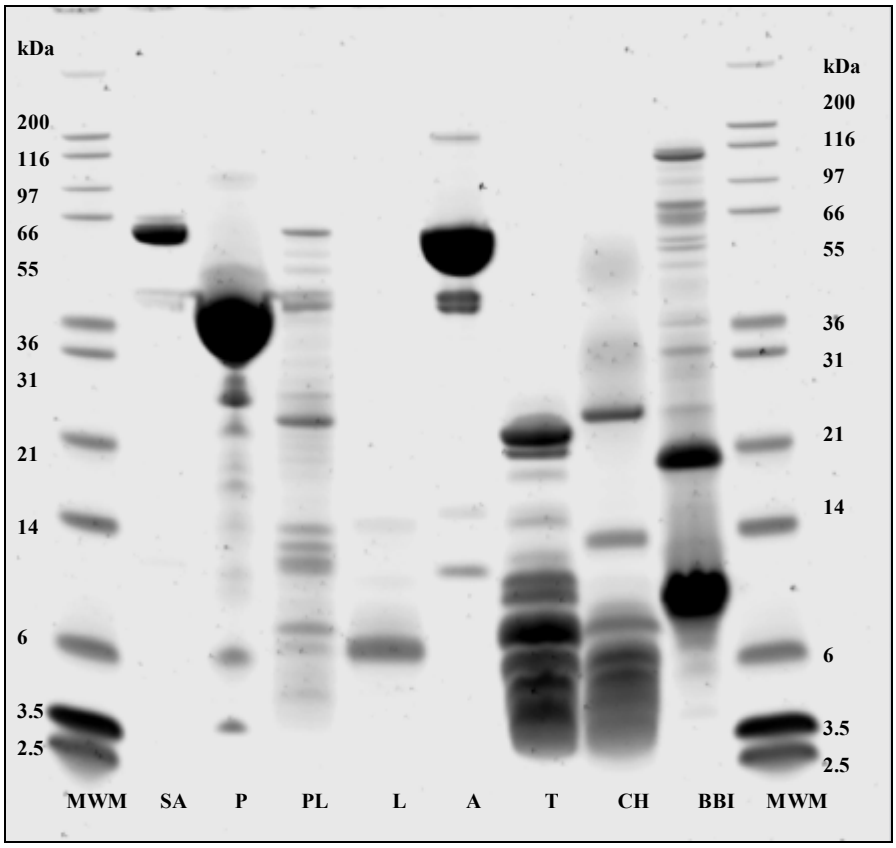

Figure 2 Gastric and duodenal digestion enzymes

MWM – molecular weight marker, SA – salivary amylase, P – pepsin, PL – pancreatic lipase, L - co – lipase, A – alpha amylase, T – trypsin, CH – chymotrypsin, BBI – Bowman-Birk trypsin-chymotrypsin inhibitor

In the comparison the gel of gastric digestion of total protein and insoluble protein (Figure 1 - Gastric A, Gastric C) we can see evident digestion of protein bands with high molecular weight range from 40 to 116000 Da. On the gel of gastric digestion of soluble proteins (Figure 1 - Gastric B) we can see spread region of digested protein in range of Mr 3500 to 30000 Da. After 3 hours of duodenal digestion we can find more bands, which were resistant to digestion by duodenal enzymes, but probably is going about bands of used enzymes. We can say, that the chemical structure of food and its physical characteristic could also limit their peptic hydrolysis, as has been shown for the *in vitro* peptic hydrolysis of gluten **(Masson *et al.*, 1989).**

The low peptic hydrolysis of bread proteins could have influenced intestinal digestion and absorption of proteins or pancreatic secretion and plasma cholecystokinin **(Guan and Green, 1996).** On the other hand, the susceptibility of some allergens to proteolysis has been reported to be altered as a result of processing and interactions between allergens and other food ingredients, particularly lipids or polysaccharides **(Moue´coucou *et al.*, 2004).** As well proteolytically resistant gluten peptides are relatively resistant to gastrointestinal digestion

and persist in the intestinal lumen, allowing them to elicit an immunotoxic response in genetically susceptible individuals **(Gass *et al.*, 2006).**

CONCLUSION

These results indicated that the subunits with low molecular weight were more resistant to proteolytic attack than the big one. However, despite hydrolysis, the remnants of the small subunit remained disulphide linked to the large one as judged by SDS-PAGE under reducing conditions. We can say that gluten proteins of high and middle molecular weight were digested on proteins of low molecular weight after gastric digestion which retain an allergenic potential.

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