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Impact of glycation on duodenal digestibility of Bowman-Birk inhibitors

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Adverse effects of soyabean-containing diets include poor digestibility and allergy to soyabean proteins. To improve the nutritional quality of soya foods inhibitors are generally inactivated by heating or eliminated by fractionation during food processing[1]. These inhibitors could generate an immune response[2]. Bowman-Birk (BBI) and Kuntiz (KTI) inhibitors are heat stable and they may be inactivated by glycation[3], which also reduces soyabean-protein allergenicity[4]. The aim of the present study was to investigate the impact of the BBI glycation on the activity of duodenal digestive enzymes in order to optimise the beneficial effects of soyabean proteins.

The BBI was glycated with glucose, fructose and fructooligosaccharides (FOS; Raftilose P95; Orafti España SL, Barcelona, Spain) in KOH (0.2%, w/v) at 60°C for 20 min. The protein glycation was confirmed by isoelectric focusing (IEF) and SDS–PAGE gel electrophoresis. *In vitro* duodenal digestion was performed using the method described by Moreno *et al.*[5]. Samples were incubated with trypsin–chymotrypsin for 120 min.

Fig. 1 shows the IEF gel (pH 3–10) stained with Comassie Blue. Different electrophoretic profiles were obtained for native and heated BBI samples (lanes 1 and 2) compared with BBI glycated with glucose, fructose and FOS (lanes 3–5). A decrease in positive charges was observed as a result of the involvement of basic amino acids (Lys and Arg) in the Maillard reaction (glycation). This analysis also confirmed BBI stability to heat treatment at 60°C for 20 min. Heating had no effect on the isoelectric point of BBI. Fig. 2 shows the SDS–PAGE gel (10%, w/v) stained with Comassie Blue. The molecular mass (8 kDa) of native BBI (lane 1) was increased as a consequence of glycation with glucose, fructose and FOS (lanes 2–4; Fig. 2(a)). All samples inhibited trypsin and chymotrypsin activities. Samples did not change their electrophoretic profiles after enzymic digestion (Fig. 2(b); lane 1, native BBI; lane 2, heated BBI; lanes 3–5 BBI glycated with glucose, fructose and FOS respectively). Further studies are needed to establish the glycation conditions that cause a reduction in BBI inhibitory activity against digestive enzymes and to identify the glycation product responsible for this change. Both IEF and SDS–PAGE analyses are suitable for following glycation of BBI and other soyabean proteins.

Fig. 1

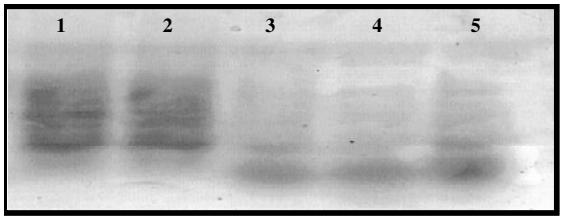
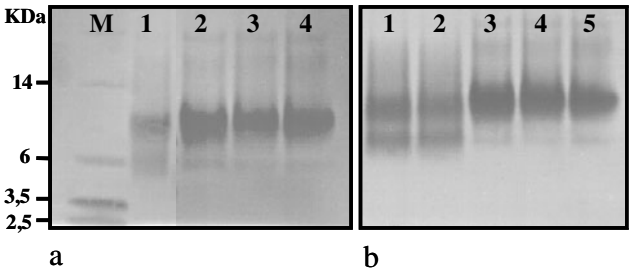


Fig. 2



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1. Friedman M & Brandon DL (2001) *J Agric Food Chem* **49**, 1069–1086.
2. Kaayla TD (2004) *Nexus Magazine* **11**; available at *www.nexusmagazine.com*
3. Kato,Y & Matsuda T (1997) *J Agric Food Chem* **45**, 3826–3831.
4. van de Lagemaat J, Silván JM, Moreno J, Olano A & Del Castillo MD (2006) *Food Res Int* **40**, 153–160.
5. Moreno FJ, Mellon FA, Wickham MS. Bottrill AR & Mills EN (2005) *FEBS J* **272**, 341–352.