In vitro enzymatic hydrolysis of protein and protein pattern change of soya and faba beans during germination

F.H.M.G. SAVELKOUL*, H. BOER, S. TAMMINGA, A.J. SCHEPERS & L. ELBURG
Department of Animal Nutrition, Agricultural University, Haagsteeg 4, 6708 PM Wageningen, The Netherlands (* author for correspondence)

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Abstract. In addition to technological processes like heat treatment, germination can be an alternative process for the improvement of protein quality of legumes. This was demonstrated by enzymatic protein hydrolysis of flour of germinated faba and soya beans, using a pepsin-pancreatin enzyme system. SDS-PAGE was used to study the changes in protein pattern of these legumes during germination. In addition, the effect of germination on the content of condensed tannins in flour from germinated faba beans and trypsin inhibitors in flour from germinated soya beans were studied. Germination for five days resulted in a maximum increase in enzymatic protein hydrolysis by 21.3% in flour from faba beans and by 25.7% in flour from soya beans after 12 hours of germination. Protein patterns, obtained with SDS-PAGE demonstrated a considerable protein breakdown during germination between day 2 and 3 in faba beans and between day 1 and 2 in soya beans. The tannin content in flour from faba beans decreased by 29.7% after seven days of germination, but the tannin content of the hulls of the faba beans did not change during that period of germination. The trypsin inhibitors in flour from soya beans decreased by 25.5% after seven days of germination. We conclude that the increased enzymatic hydrolysis of protein in both legumes cannot be explained by a decrease of tannins or trypsin inhibitors. The possible explanation is that through degradation of proteins during germination of the legumes, the cleaved protein fragments are more susceptible for hydrolysis by pepsin-pancreatin.

Introduction

Native storage proteins in ungerminated legume seeds like phaseolin in white kidney beans (Phaseolus vulgaris) are quite resistant against proteolytic degradation in the digestive tract of monogastric animals like pigs and poultry [1]. Storage proteins in ungerminated legume seeds like legumin and vicilin in faba beans (Vicia faba) and β-conglycinin and glycinin in soya beans (Glycine max) can possibly have the same effect as phaseolin in monogastric animals. True digestibility of raw faba and soya beans appear
to be relatively low [2, 3]. Even when this is taken into account the apparent protein digestibility is lower than expected because of the presence of antinutritional factors such as protease inhibitors (trypsin-/chymotrypsin inhibitors), tannins and lectins [4, 5, 6]. In order to improve the protein quality of legumes much research aiming at the elimination of antinutritional factors, often of technological nature, has started in recent years [7, 8]. Apart from technological treatments there seems scope for alternative processes. One promising area of biotechnological research aiming at the improvement of protein quality of legumes is germination [9]. Wassef et al. [10] concluded that proteolysis of protein in soya bean meal after treatment with papain and trypsin was increased when the meals were defatted, prepared from germinated soya beans or digested for long periods. They also found that germination and seedling growth of soya beans were accelerated by impaction, soaking in water and growth at a temperature of 30°C. Rahma et al. [11] studied the effect of germination on the in vitro protein digestibility of faba beans. Using pepsin-pancreatin as well as pepsin, pancreatin and trypsin as single enzyme systems they concluded that the in vitro digestibility increased up till 4 days of germination.

In order to investigate the effect of germination on in vitro enzymatic hydrolysis of bean protein an experiment was conducted in which faba beans and soya beans with a relatively high content of tannins and trypsin inhibitors, respectively, were germinated for up to 7 days. Total crude protein content, water absorption, enzymatic hydrolysis of protein and protein pattern of flour from faba and soya beans were measured. In addition the effect of germination on the content of tannins and trypsin inhibitors was measured in bean flour from faba beans and soya beans, respectively. Finally, during germination the tannin content of the hulls of faba beans was measured in order to compare results with the content of the tannins in the bean flour.

Materials

Faba beans (Vicia faba, var. Pistache) were obtained from Joordens Zaadhandel BV, Neer, The Netherlands; soya beans (Glycine max) were obtained from Schouten-Giessen NV, Giessen, The Netherlands.

Germination

All bean varieties were germinated in wet sand under standard conditions (12 hours light/day and 20°C) at the former Government Seed Testing Station (RPvZ), Wageningen [12]. Germination was stopped by harvesting after 0, 0.5, 1, 2, 3, 4, 5, 6 and 7 days.