Effects of Processing on Bean (*Phaseolus vulgaris* L.) Protein Quality

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ERRATUM: Table 6, page 97: replace (k_r, sec^{-1}) by (k_r, hr^{-1})

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STELLINGEN

I.

Potentiële verbetering van de verteerbaarheid van peulvruchten door middel van procesbehandelingen wordt door de veevoederindustrie slechts ten dele benut.
(Dit proefschrift).

II.

Lage lectine gehalten in warmtebehandelde *Phaseolus* bonen zijn geen garantie voor de hoogst mogelijke voederwaarde.
(Dit proefschrift).

III.

De bepaling van het gehalte van lectinen via ELISA is meer onderscheidend dan de bepaling van de haemagglutinatie-activiteit.
(Dit proefschrift).

IV.

Voor het beperken van het aantal dierproeven is onderzoek naar en verbetering van de *in vitro* enzymatische methoden noodzakelijk.
(Dit proefschrift)

V.

Het door Rackis *et al.* aangehaalde effect van vocht op de inactivering van enzymremmers in zogenaamde 'soja'bonen geeft aan dat identificatie van bonen niet alleen bij hun verwerking maar ook in de wetenschappelijke literatuur een probleem kan zijn.
(Rackis *et al.*, 1986; In: *Advances in Experimental Medicine and Biology Series*: M. Friedman [Editor], Plenum Press, New York, 299-347).

VI.

In de discussie ten aanzien van kippen op legbatterijen en scharrelkippen dient ook de gezondheid als voorwaarde voor het welzijn betrokken te worden.

VII.

Procestechnologie biedt betere mogelijkheden om de voederwaarde van ruwvoeders voor herkauwers te verbeteren dan enzymtechnologie.

VIII.

Improved 'feed conversation' cannot be an ultimate objective of soya bean processing.
(Walter, 1988; *Feed Magazine*, nov./dec, 47-50).

IX.

Honger maakt rauwe bonen zoet maar niet minder toxisch.

X.

In vergelijking met analoge, modulaire systemen doen digitale synthesizers afbreuk aan de creativiteit van de componist.

XI.

Nagelbijten kan geestelijke schade door frustratie voorkómen.

XII.

Alcohol-vrij bier is alleen goed voor de dorst.

Stellingen behorende bij het proefschrift "Effects of processing on bean (*Phaseolus vulgaris* L.) protein quality".

A.F.B. van der Poel

Wageningen, 8 juni 1990

Voor Ine, Rob

mijn ouders
mijn schoonouders

VOORWOORD

Dit proefschrift bestaat uit een zestal wetenschappelijke artikelen, waarvoor het onderzoek is uitgevoerd bij de vakgroep Veevoeding van de Landbouwuniversiteit te Wageningen. Een deel van de experimenten is uitgevoerd in samenwerking met de vakgroep Levensmiddelentechnologie, sectie Proceskunde, met het TNO Instituut voor Voeding en Fysiologie van Landbouwhuisdieren (ILOB) te Wageningen en de vakgroep Pathologie van de Rijksuniversiteit te Utrecht.

Een groot aantal personen heeft een directe of indirecte bijdrage geleverd aan het tot stand komen van dit proefschrift. Voor haar of zijn bijdrage dank ik een ieder zeer hartelijk. Josien van de Kraats (het vlotte en correcte typewerk; de layout van het proefschrift), Karel Boekhorst (de tekeningen en grafieken), Willem Stolp (het ziften, het toasten) en alle (ex-)studenten wil ik op deze plaats graag noemen.

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INTRODUCTION

Animal feed manufacturing involves the use of a variety of raw materials to produce complete diets. These diets are defined according to nutritional and technological specifications to meet the requirements of several animal species. Some raw materials, for example certain legume seeds, can satisfy these specifications only after some form of processing.

Feed legumes represent a diverse assemblage of plant seeds suitable as ingredients for animal feeds. Pea (*Pisum sativum*), fababean (*Vicia faba*), common bean (*Phaseolus vulgaris*), lupin (*Lupinus* spp.), soya bean (*Glycine max*), lentil (*Lens culinaris*) and chickpea (*Cicer arietinum*) are considered legume crops capable of producing good yields in the temperate climate common to much of Europe (Wiseman and Cole, 1988).
The genus *Phaseolus* includes all species of legume seeds normally known as common beans. Within the group of common beans various bean-type designations are distinguished (Great Northern, Small White, Navy, Kidney, etc.). These beans can vary considerably in shape, size and colour.
Diet ingredients for livestock supply energy yielding and other nutrients which are essential for an efficient and economical livestock production. In common bean, protein and carbohydrates are the principal nitrogen and energy-yielding components, respectively. When compared with animal products the nutritive values of beans, particularly proteins, are often lower than is expected from their chemical analysis. The nutritive values are limited in a number of aspects (Sgarbieri and Whitaker, 1982):

a. amino acid composition of the proteins, availability of amino acids and digestibility of proteins.
b. the presence of toxic proteins and other so-called antinutritional factors.

Antinutritional factors (ANF) are referred to as constituents which are naturally present in many types of dry legume seeds (Liener, 1980; Chubb, 1982). They form a plant defense mechanism against insect, microbial and bird predation (Jansen *et al.*, 1976). The ANF which may influence the protein utilization of common beans are mainly associated with lectins (or phytohaemagglutinins) and protease inhibitors (Pusztai and Palmer, 1977; Liener, 1980) and in some varieties also with polyphenols (Reddy *et al.*, 1985).

Bean lectins constitute a class of (glyco)proteins with the ability to combine with glycoconjugates located in the mucosa of the intestine. They are resistant to gut proteolysis to a considerable but variable extent (Liener, 1980; Pusztai, 1987). Due to their binding to glycoconjugates in the epical membrane of the epithelial cells which line the small intestine, lectins may account for various morphological and functional changes in the epithelium of the small intestine in animals (Pusztai, 1987; Kik *et al.*, 1989). Lectins may therefore interfere with digestion and absorption of nutrients. They may cause growth depression in the animal and may even become lethal. This effect of lectins on animals is different from the effect of other ANF such as protease inhibitors. The growth depression effects of the latter inhibitors are thought to be due to a decrease in proteolysis of feed proteins in the intestinal lumen. As a consequence, digestion of dietary protein may be reduced. In addition, other mechanisms which cause pancreatic hypertrophy and hyperplasia, presumably due to the overstimulation of pancreatic enzyme secretion, are involved (Liener, 1980; Burns, 1987).

In addition to ANF, the *Phaseolus* bean protein itself is somewhat resistant to enzymatic attack; the digestibility of the raw protein, therefore, may be low. It is notable that the contribution of lectins, protease inhibitors and the storage protein of beans to the negative effects on feed digestion may vary with the differences in the gastro-intestinal tract of several animal species.

Piglets, rats and chicken have been observed to react differently (Table 1) during short-time feeding of (un)processed *Phaseolus* bean diets (Huisman *et al.*, 1990; Van der Poel *et al.*, 1990a,b). From these recent studies it appears that the pig is the most sensitive species to the negative effects of the ANF present in raw or underheated *Phaseolus vulgaris*, followed by the chick and than the rat (Table 1). It was indicated that the pig digests raw protein with much greater difficulty than the rat (Van der Poel *et al.*, 1990b). Also, the growth of the small intestine was enhanced, an effect which is no doubt due to the binding of the lectin (King *et al.*, 1983). Because the diets used in these studies contained the whole bean, conclusions are difficult with respect to the contribution of each separate ANF (lectins; protease inhibitors) to the relative sensitivity of different animals to their biological effects.

Most of these negative effects could be compensated effectively by that degree of heat treatment which causes inactivation of lectins and trypsin inhibitors. This may be taken as a preliminary evidence for these compounds as major causative factors. In addition, however, the bean protein itself may undergo significant changes owing to denaturation induced by thermal treatments.

On the basis of these results and because plant proteins are often used in diets for pigs, it was decided to use the piglet as the target animal for *in vivo* evaluation of heat treated beans.

Table 1. Feed intake (g/d), daily gain (g/d) and feed conversion efficiency (FCE) of piglets, chicken and rats after 14 days of growth.[1]

	Control diets	Experimental diets[2]			
		T0	T20	T40	T80
Rats					
Feed intake	12.3	12.3	12.5	12.4	12.4
Daily gain	2.99	2.92	3.15	3.13	3.08
FCE	4.19	4.26	3.98	3.97	4.05
Chicken					
Feed intake	17.2^{a}	16.7^{b}	17.3^{a}	17.2^{a}	17.2^{a}
Daily gain	9.6^{a}	8.7^{b}	10.0^{a}	10.0^{a}	9.9^{a}
FCE	1.79^{a}	1.92^{c}	1.73^{b}	1.73^{b}	1.75^{ab}
Piglets					
Feed intake	233.9^{a}	134.8^{b}	214.3^{a}	225.4^{a}	225.7^{a}
Daily gain	151.6^{a}	-3.5^{b}	121.5^{c}	145.8^{a}	138.0^{a}
FCE	1.55^{a}	--	1.81^{b}	1.56^{a}	1.65^{a}

[1]Data from Van der Poel *et al.* (1990a,b).
[2]T0, T20, T40 and T80 refers to bean thermal processing at 105°C during 0, 20, 40 and 80 min., respectively.
[a,b,c]Values with different superscripts in the same row differ significantly ($P<0.05$)

Commercially available *Phaseolus* seeds cannot always be distinguished according to their correct botanical names. This problem of identification is further aggravated by contamination of other commercial samples (Grant *et al.*, 1983). Therefore, available batches of beans cannot be labelled according to their ANF properties. Since the lectins from *Phaseolus* beans are considered potentially toxic, as a routine, beans of *Phaseolus* are always processed prior to the inclusion into diets.

Feed manufacturing involves the (primary) processing of separate dietary ingredients as well as the (secondary) processing of the complete diets. These technologies can provide feed intake and utilization of nutrients which are more optimal than without these treatments. Techniques for primary processing, currently applied on animal feed ingredients are steam treatment, infrared irradiation and extrusion. In addition to these thermal treatments, new techniques e.g. dry fractionation (Vose *et al.*, 1976) and the use of chemicals (protein modification by disulfide interchange; Friedman and

Gumbmann, 1986) have been explored. These techniques cannot always be applied in routine procedures for the manufacturing of complete animal diets. Specific processing conditions have to be used to control the treatment in the best possible way. Therefore, techniques for primary processing are generally applied in specialist plants.
The upgrading of beans by processing is largely based on thermal treatment, which is an effective way to decrease the activity of protease inhibitors (Rackis *et al.*, 1986) and lectins (Antunes and Sgarbieri, 1980). In addition, a positive effect of heat treatment on the *in vitro* digestibility of the major storage protein has been suggested (Deshpande *et al.*, 1983).
The effectiviness of thermal treatments in establishing an improved nutritional value depends on a combination of process temperature, time of exposure, particle size and moisture content. In controlling the final product quality, processing must be carried out under well-controlled and standardized conditions. Moderate conditions of heating are often necessary for improving nutritional quality. Overheating, however, will diminish protein quality through a decreased availability and/or content of lysine (Almas and Bender, 1980). Also, it may cause a slower release of amino acids from the protein (De Wet, 1982). Therefore, equipment is needed to measure process characteristics precisely, in order to define the optimal thermal treatments.

A general aim of protein nutrition is that all amino acids can serve protein metabolism in the animal as far as possible (biological value). This can only be achieved by elimination of lectins which are major inhibitors of protein utilization in *Phaseolus* beans (Grant *et al.*, 1983; Pusztai, 1987). Elimination of lectins (a protein itself) most likely also eliminates protease inhibitors. This does, however, not necessarily mean that after elimination of these ANF, the storage protein can be used maximally by the animal. *In vitro* measurements have been suggested not to reflect the actual physiological state of the intestine. Consequently, these estimates cannot be considered as substitutes for biological appraisal of protein quality (Bender, 1984; Donnatucci *et al.*, 1988). Upgrading of beans, therefore, must include at least short-term feeding experiments to relate nutritional parameters and *in vitro* indicators for a precise estimate of the effectiveness of a process.

The aim of the studies described in this thesis is to investigate the effect of different processing conditions of beans on the residual level of anti-nutritional factors and on *in vivo* digestibility of the resulting products. Steam processing technology has been chosen as a principle process for heat treatments.

General aspects of lectins, trypsin inhibitor activity and protein nutritional value in beans of *Phaseolus vulgaris* in relation with processing are reviewed in Chapter I. Examination of the literature clearly shows that research efforts to establish maximum nutritional value of beans for animals through processing are mostly based on heat treatment. These efforts are directed mainly towards the elimination of antinutritional factors. The effects of heat treatments on essential nutrients have been hardly studied. A great variation of processes have been used. Only few systematic studies of the process have been made which have been followed by evaluation with a biological assay.

Different types of ANF have been identified in different seed fractions. Therefore, fractionation seemed to be a first logical step in removing ANF from various feed components. In Chapter II a fractionation technique for the possible separation of proteinaceous ANF from storage protein is described. For that purpose, fine milling of beans and subsequent air classification was used to fractionate beans into its main constituents. The effect of the type of milling as well as changing the processing variables for air classification on the distribution of proteins, lectins and trypsin inhibitor activity was evaluated. Preliminary experiments showed that variation in processing characteristics during air classification did affect the composition of proteins in various fractions (Patel *et al.*, 1980; Sosulski *et al.*, 1987). The effect of lectins on cultured mucosal tissues of pig small intestine was verified and also sensitive assays were used to determine the level of total and functional lectins (Hamer *et al.*, 1989).

In studies of thermal treatments of feedstuffs only a few temperatures at various duration times have been applied according to literature. We decided to use a technique in which temperature and time can be altered easily and accurately. It was further decided to compare High Temperature Short Term (HTST) and Low Temperature Long Term (LTLT) processing. A pressurized steaming equipment was modified for this purpose to ensure sufficient controls to minimize ambiguities (Chapter III). In this approach, processing variables can be evaluated by deriving reaction kinetics. This approach, therefore, combines the features of a more fundamental and a practical assessment of different heating procedures.

In Chapter IV the products derived from steam processing of beans are evaluated by chemical methods. It was decided to assess the influence of steaming both at ~100°C and at two high temperatures (~120 and ~140°C) in a HTST-designed process for different processing times. The products resulting from these treatments will differ in the content of antinutritional

factors (level and activity of lectins and protease inhibitors). Kinetic aspects of the inactivation reactions of ANF are discussed as well as kinetics of nutrient (lysine and available lysine) retention and of protein solubility.

Heating of a feed ingredient will affect not only ANF, also the nutrients may be altered in such a way that their components or availability for the animal is affected (Gall, 1989). Therefore, the consequences of the thermal treatments for potential nutritional value of the total bean proteins were studied with *in vivo* techniques (Chapter V) and *in vitro* techniques (Chapter VI).
For physiological consequences of various processing methods, ileal digestiblity is thought to be a good estimate. There is no literature available on the ileal digestibility of heat processed *Phaseolus* beans nor wether the digestibility is influenced by the temperature used in the processing. In Chapter V the implications of different steam processing conditions on the ileal digestiblity of protein and lysine are described. The effect of bean inclusion level in the diet was also investigated.

A major drawback in *in vivo* experiments determining digestibility by total collection of ileal chyme or faeces is the requirement of large amounts of feeds. Also, these methods are time consuming and only a limited number of feed samples can be tested. Since many processing variables need to be evaluated, more rapid feed evaluation techniques have been proposed such as *in vitro* enzymatic digestibility assays (see Moughan *et al.*, 1989; Babinszky *et al.*, 1990) and the Mobile Nylon Bag Technique (MNBT; Sauer *et al.*, 1983). Chapter VI deals with a series of experiments in which the protein digestibility was determined according to such rapid evaluation techniques.

The experiments described in this thesis were performed with the aim to contribute to the evaluation of processing plant feedstuffs to be used for animal production. A lot of feedstuffs have to be evaluated and processing of samples implies the evaluation of a large number of treated products. In order to keep it managable we resticted research to beans of *Phaseolus vulgaris*. This is a typical example of a plant protein with a potential for increased nutritive value after steam processing.

In the General Discussion the aim of processing and its consequences for protein quality parameters are discussed. Firstly, the processes need to be evaluated technologically. Secondly, the aim of processing a feedstuff should be to maximize nutrient availability of the feedstuff. The study of this involves many subsequent steps. Therefore, techniques, which can be used to diminish the number of steps or from which information is gained more

rapidly, should be employed as much as possible. The evaluation of these latter steps in connection with process variables is of real importance for improvement of processing practices and optimizing nutritional value.

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CHAPTER I

Effect of Processing on Antinutritional Factors (ANF) and Protein Nutritional Value of Dry beans (*Phaseolus vulgaris* L.). A Review.

A.F.B. van der Poel

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Abstract

Seeds of common beans (*Phaseolus vulgaris* L.) contain several antinutritional factors (ANF). For example, they contain some heat-sensitive proteins that greatly reduce the nutritional value of unprocessed beans in feeding monogastric animals.
Proteinaceous inhibitors require their structural integrity for their inhibiting activity; therefore, heat processing abolishes the negative effects by denaturing these proteinaceous inhibitors.
The increase of the *in vivo* protein nutritional value of beans after heat processing, however, does not allow the elucidation of the nutritional significance of a specific ANF. This is due to the simultaneous presence of other ANF which still exert (heat- stable) or do not exert (heat-sensitive) their negative effects after processing. There seems to be only little correlation between residual activity of ANF in heat-treated beans and *in vivo* protein nutritional value. For estimating a more precise bean protein nutritional value after processing more detailed information is required on residual ANF-activity, based on functional ANF assays in relation with target animals. Moreover, nitrogen partitioning in feed or endogenous origin has to be elucidated.

Introduction

Seeds of the family *Leguminosae*, referred to as pulses, feed legumes, dry beans, etc., are important sources of proteins, minerals, vitamins and energy in diets for especially monogastric animals. Nevertheless, their role in animal nutrition could be even more important if several factors can be removed. These limiting factors of dry beans are associated with:

1) low protein quality associated with low proportions of sulphur amino acids, a low inherent protein digestibility and/or antinutritional factors, respectively.
2) flatulence and general gastro-intestinal distress experienced after ingestion of legume products and associated with e.g. oligosaccharides.

Many different antinutritional factors (ANF) are found in raw legumes (Liener, 1980) and they include a wide range of digestive inhibitors, toxins and other substances interfering with appetite, absorption or metabolism in various ways. Many of these ANF can be inactivated by adequate heat treatment, such as might be employed in primary processing procedures for livestock feeding purposes. The nutritional significance of the residual ANF-activity or enhanced protein digestion after processing, however, has not

been properly assessed in view of the various practical processing applications for livestock feeding.
It becomes important, therefore, to study the removal, elimination or inactivation through processing of the inhibitory constituents and coherent factors in legume seeds, necessary to enhance protein digestion *in vivo*. By elucidating the *in vivo* responses of the animal to processed beans one might be able to establish the processing conditions and quality control criteria necessary to optimize bean treatments for livestock consumption.

This paper reviews the available information on the reduction by heat treatments of antinutritional and coherent factors in dry beans of *Phaseolus vulgaris* and the consequences of thermal treatments for its protein nutritional value in pigs.

Protein quality of beans

Cultivated common beans *(Phaseolus vulgaris)* are a large group of several genetic varieties in this species. Various bean-type designations are distinguished such as Navy, Great Northern, Pinto, Kidney, Small Red, etc. The protein in beans is located mainly in the cotyledon and in the embryonic axis of the seeds. Small amounts are also present in the testa. On a dry weight basis the beans contain less than 2% lipid and approximately 5% crude fibre, 4-6% ash and up to 60% total carbohydrate. Starch is the major component of the carbohydrate fraction (Tobin and Carpenter, 1978; CVB, 1988).
Based on N x 6.25 as the conversion factor, crude protein content generally ranges between 20 and 27% on a dry weight base. However, nitrogen to true protein conversion factors based upon the nitrogen content of amino acids in feed legumes range from 5.38 to 5.86 (Peace *et al.*, 1988) indicating that the use of the 6.25 factor overestimates the true protein content of legumes.
The nutritional value of protein in *Phaseolus vulgaris* bean is low and varies between species and between cultivars (Sgarbieri, 1979). This is mainly due to a deficiency in sulphur amino acids and the low protein digestibility (Bressani and Elias, 1978; 1988). The nutritional properties of common bean proteins are shown in Table 1.
Faecal or ileal apparent nitrogen digestibility, measured by *in vivo* experiments, is low. Also, after heat processing crude protein digestibility is lower than that of casein (Rockland and Radke, 1981; Koehler *et al.*, 1987) and the availability of essential amino acids may be depressed after heating.
The relative low digestibility is attributed to the resistance of the major storage protein (phaseolin) to proteolysis and to the presence of fibre, tannins

Table 1. Some general nutritional properties of bean (*Phaseolus vulgaris L.*) proteins

Amino acid composition
Deficient in sulphur amino acids (methionine, cystine, cysteine), particularly in main storage globulins.
High concentration of lysine.
Amino acid levels (g/100 g protein)[1]:
Methionine : 0.57-0.94
Cysteine : 0.12-0.40
Tryptophan : 0.80-1.00
Lysine : 4.70-6.40
Digestibility
Relatively low compared to other legumes
Depending on content of pigment in testa (polyfenols)
Resistance of protein globulin components to proteolytic digestion
Affected by antinutritional factors
Biological availability of amino acids can be low after processing because of
(1) substantial numbers of disulfide bonds in proteins
(2) Maillard type reactions upon processing.

[1]Data from Sgarbieri and Whitaker (1982).

and other polyphenolic compounds (Philips *et al.*, 1981; Aw and Swanson, 1985) in dry seeds. Protein utilization of common beans is also negatively affected by constituents like lectins, protease inhibitors and other inhibitors (Liener, 1980).

These antinutritional factors are present at relatively small or substantial (lectins) levels. They should be taken into account evaluating bean protein quality, even though it is the protein itself which is the component of primary interest.

Only feed legumes that contain low levels of deleterious ANF can be evaluated in the raw state. Appropriate heat processing causes destruction or inactivation of some of these ANF. This is necessary to determine the true protein value of beans which cannot be fed in the raw state.

Factors influencing bean protein utilization

The efficiency of the utilization of proteins from legume seeds depends on, firstly, the inherent protein resistance to proteolysis and, secondly, the interference by antinutritional factors.

Resistance to proteolysis

Seeds of most cultivars of the common bean *(Phaseolus vulgaris L.)* contain similar constituent protein fractions based on solubility properties (Andreas *et al.*, 1986): phaseolin (36-46%, by weight), globulin-2 (or G2/albumin) (5-12%), albumin (12-16%), prolamine (2-4%) and an alkali-soluble fraction (20-30%). The protein distribution and composition in cultivars of *Phaseolus vulgaris* is shown in Table 2.

Table 2. Protein distribution (% of total), protein (%) and amino acid (g/100g protein) composition of seeds from *Phaseolus vulgaris* L.[1]

Sample(s)[2]	Protein distribution			Protein composition				
	Lectin	Phaseolin	NLNP[3]	Level	Amino acid profile			
					Lys	Meth	Cys	Trp
Sanilac	12.7	41.3	45.9	19.7				
L/L	11.9	46.9	41.2	21.4				
l/l	0.0	60.7	39.3	22.2				
25 varieties				24.1	8.46	1.17		0.68
6 cultivars					7.90	0.50	0.71	
100 lines				22.7		1.12	0.98	
120 varieties				25.2	7.31	1.10	1.29	

[1]Data adapted from Sgarbieri and Whitaker (1982) and from Osborn and Bliss (1985).
[2]L/L and l/l are lectin-containing and lectin-less backcross lines.
[3]NLNP = Nonlectin nonphaseolin protein.

Phaseolin (globulin) is the major storage protein of dry beans. The resistance to proteolysis of phaseolin, has been considered a main reason for their poor nutritional value (Kakade *et al.*, 1964; Liener and Thompson, 1980; Chang and Satterlee, 1981; Sathe *et al.*, 1983). The theories proposed for the phaseolin resistance to proteolysis found in *in vitro* experiments are controversial, as discussed by Deshpande and Nielsen (1987b). The techniques of measuring *in vitro* protein digestibility are not standardized very well.

Table 3. Antinutritional factors associated with the digestion/utilization of bean protein and carbohydrates in monogastric animal nutrition.

Antinutritional factor	Major in vivo effect	References
Lectins	Damage of gutwall	Donatucci, 1983; Kik *et al.*, 1989 Donatucci *et al.*, 1987
	Immunological reaction	Greer, 1983; Liener, 1986;
	Metabolism toxicity	Pusztai, 1987
Protease inhibitors		
Trypsin/chymotrypsin inhibitor	Reduction activity of (chymo)trypsin	Liener, 1979; Richardson, 1980/81; Sgarbieri and Whitaker, 1982; Burns, 1987; Birk, 1989
	Pancreas hypertrophy	
	Decreased digestion	
α-Amylase inhibitor	Forming complex with amylase in salivary and pancreatic juice	Powers and Whitaker, 1977
	Reduces starch availability	
Tannins and polyphenolic compounds	Forms complex with enzymes or feed protein	Griffith, 1981; Philips *et al.*, 1981; Aw and Swanson, 1985; Marquardt, 1989
	Reduces protein digestibility	
Flatulence factors	Gastrointestinal discomfort	Fleming, 1981; Fleming *et al.*, 1988
	Increased maintenance	
Fibre-protein	Proteolysis hampered	Murphy *et al.*, 1972; Fleming, 1981
Phytic acid	Forms complex with anions and protein	Reddy *et al.*, 1982, Lolas and Markakis, 1975
	Depresses absorption of minerals	
Storage proteins		
Quality	Low digestibility	Sgarbieri and Whitaker, 1982
Resistance to proteolysis	Low digestibility in gastro-intestinal tract	Kakade *et al.*, 1964; Liener and Thompson, 1980; Deshpande and Nielsen, 1987a, 1987b
	Pancreas hypertrophy	

Antinutritional factors (ANF)

Dry beans of *Phaseolus vulgaris* and other legume seeds contain a variety of constituents which can interfere with appetite, absorption and metabolism. A survey of these factors and their negative effects are given in Table 3. In many cases, the levels of antinutritional factors are not considered to be absolute and may vary depending on -firstly- the variety and, furthermore, on cultivar, climatic conditions, location etc.

The contribution of these antinutritional factors to the poor nutritional quality of raw *Phaseolus* beans has been associated with lectins (Donatucci, 1983; Greer, 1983; Liener, 1986; Pusztai, 1987), protease inhibitors (PI; Rackis *et al.*, 1986), amylase inhibitors (Marshall and Lauda, 1975; Powers and Whitaker, 1977), phytate (Reddy *et al.*, 1982), tannins and polyphenols (Salunkhe *et al.*, 1982; Aw and Swanson, 1985) and oligosaccharides (Fleming *et al.*, 1988). Reviews on these ANF have been made by Liener (1980), Sgarbieri and Whithaker (1982), Chubb (1982), Bondi and Alumot (1987) and Gupta (1987). A considerable amount of work has been conducted on specific biochemical aspects of various ANF without direct application to the utilization of legume seeds for animal feed. This work has included studies on the isolation, identification, characterization and analysis of individual constituents such as lectins, protease and α-amylase inhibitors. As pointed out by Richardson (1980-81) and Gupta (1987) a notable feature of these inhibitors is the heterogeneity for their levels in *Phaseolus* beans, with differences between bean-type designations and even between cultivars.

Primarily lectins and also protease inhibitors (PI) in *Phaseolus vulgaris* are the ANF considered the most important. More recently the importance of tannins and associated polyphenolic compounds has been recognized (Fernandez *et al.*, 1982). In the following some specific ANF are briefly described for *Phaseolus vulgaris*. For the proteinaceous ANF, some properties are summarized in Table 4.

Lectins constitute a class of proteins which are characterized by their unique ability to bind to glycoprotein and carbohydrate. Lectin protein in Kidney bean is made up of five tetrameric proteins (isolectins; Goldstein and Poretz, 1986). The isolectins are derived from combinations of two slightly different subunits, one erythrocyte-reactive (E) and the other leucocyte-reactive (L) (Felsted *et al.*, 1982). In this way, a leukoagglutinin (L-PHA; four identical L-subunits), an erythroagglutinin (H-PHA; four identical E-subunits) and three mitogenic isolectins containing various proportions of L and E subunits (L3E, L2E2 and LE3) are distinquished.

Lectins have been shown to be a strong growth inhibitor in *Phaseolus vulgaris* (Grant *et al.*, 1983; Pusztai *et al.*, 1981) in diets for rats (Honovar *et al.*, 1962) and pigs (Myer *et al.*, 1982; Huisman *et al.*, 1987; Van der Poel and Huisman, 1988). Lectins, (glyco)protein in nature, interact with the glycoproteins on red blood cells (causing agglutination of the cells) and with glycoproteins in mucosa of the intestinal wall. This may reduce the extent of feed digestion and absorption and may increase cellular protein and mucin synthesis. Lectins influence systemic metabolism and immune system and catabolic breakdown of body tissue (Pusztai, 1987). Grant *et al.* (1983) showed that the toxic factor from *Phaseolus vulgaris* was identical with its constituent lectins.

Table 4. Properties of some proteinaceous ANF from *Phaseolus vulgaris* L.[1]

	Proteinaceous ANF		
	Lectins[2]	Protein inhibitors of Serine protease	α-amylase
Occurence/ Distribution	2-15% of total protein Cytoplasm of cotyledons and embryonic cells	0.2-2% of total protein Cotyledons (85%) not in embry	± 0.20% Present in most cultivars Concentrated in cotyledons
Nature	Glycoprotein; subdivision in units Low content of S-amino acids	Protein, no carbohydrate High content of S-amino acids	Glycoprotein with 8-10% carbohydrate
Molecular form	High degree of heterogeneity MW 114.000-150.000 subunits: 29.000-36.500	Multiple forms *arginine-like (AL) *lysine-like (LL) MW 8.000-10.000	High degree of heterogeneity
Activity	Haemagglutinating and/or mitogenic activity	Decrease activity trypsin (AL) or chymotrypsin and trypsin (LL)	

[1]Adapted from Richardson (1980-1981), Sgarbieri and Whitaker (1982) and Brown *et al.* (1982)
[2]Variously referred names are: lectins, phytohaemagglutinins, phytolectins, G2.

Bean varieties vary in the amount and toxicity of their lectins. The lectin properties from cultivar to cultivar may vary more than between bean-types and the colour of the seed coat (Jaffe, 1980; Scarbieri and Whitaker, 1982). The inhibition of growth may be termed a toxic factor. The level of toxicity is directly related to the lectin content and hence haemagglutinating activity (Pusztai and Palmer, 1977). The highest activities of lectins are found in the albumin fraction with considerable activity present also in the globulin fraction (Antunes and Sgarbieri, 1980).

Lectins are present in relatively large quantities in the seeds of *Phaseolus vulgaris* and make up approximately 2-15% of the total seed protein (Sgarbieri

and Whitaker, 1982; Donnatucci, 1983; Greer, 1983; Osborn *et al.*, 1984). The contribution of lectins to the poor nutritional quality of raw beans has attracted much attention, resulting in numerous studies and reviews on their physical and chemical properties (Liener, 1976; Lis and Sharon, 1981; Sgarbieri and Whitaker, 1982).

It is clear that lectins are specific compounds and several distinct lectins and isolectins have been isolated. However, these substances have chemical resemblance and are identified by haemagglutinating activity (Grant *et al.*, 1983). Heat denatures proteinaceous molecules and, subsequently, the activity of lectins is decreased after heating, but often not entirely eliminated.

Protease inhibitors (trypsin-chymotrypsin inhibitor) have been extensively studied. They form a group of proteins which inhibit proteolysis of feed protein by inhibiting the action of enzymes (trypsin, chymotrypsin, amylase) in the small intestine (Sumathi and Pattabiraman, 1976). These inhibitors are also important in another aspect. They form a small part of the bean protein (2.5%) but 30-40% of the cystine of the bean protein is associated with the inhibitor (Kakade, 1974). Thus, trypsin inhibitor in its inactivated form can potentially serve as a source of the sulphur-containing amino acids. Almquist *et al.* (1966) found that cystine is not easily released when incubated with proteases.

Levels of trypsin inhibitor activity in *Phaseolus vulgaris* in a range of legume seeds are ranked second after soybeans (Gallardo *et al.*, 1974). There is evidence which suggests that the protease inhibitors in common beans do not play a major role as a growth inhibitor (Liener and Thompson, 1980; Philips *et al.*, 1981). The highest TI activities in *Phaseolus vulgaris* were found in the albumin fraction of the protein. Protease inhibitors are of low molecular weight and are well characterized (Sgarbieri and Whitaker, 1982). Some properties are indicated in Table 4.

There are two types of protease inhibitors found in common beans. They include the inhibitors of the serine group (chymotrypsin and trypsin) and of the α-amylases. The Bowman-Birk soybean protease inhibitor appears to be a good prototype of the *Phaseolus* protease inhibitors (Jacob and Pattabirama, 1986). Inhibitors from *Phaseolus* do not contain carbohydrate and have multiple forms (Wilson and Laskowski, 1973; Richardson, 1977; Sanderson *et al.*, 1982; Tsukamoto *et al.*, 1983a). Three (Wilson and Laskowski, 1973) and five (Tsukamoto *et al.*, 1983b) different inhibitors have been purified from kidney beans, having almost identical properties.

Comparable to lectins, the trypsin/chymotrypsin inhibitors are inactivated by denaturation after heat treament.

Jaffe *et al.* (1973) found α-amylase inhibitor in 79 of 95 legume varieties tested. The most active extracts were from kidney beans. The amylase

inhibitor in red kidney beans has been indicated as the factor to be responsible for impaired digestion of starch (Jaffe and Vega Letta, 1968). Subsequent work, however, has shown that the addition of a purified inhibitor to various diets did not affect the availability of starch in rats (Savaiano *et al.*, 1977).

Tannins and associated polyphenols which are present in feed legumes have been investigated only recently compared to previous mentioned factors. The tannins are located mainly in the seed coat and have been found to adversely affect protein digestibility in coloured cultivars (Elias *et al.*, 1979). It has been suggested that the level of tannins is related to flower colour. Studies show that white seeded varieties contain negligible amounts of tannins, while coloured seeded varieties contain large quantities of tannins (Bressani and Elias, 1978; Reddy *et al.*, 1985).

Condensed tannins are able to form complexes with proteins, including enzymes, and may reduce digestibility of protein and activity of enzymes in diets for nonruminants. Sathe and Salunkhe (1984) suggested a non-specific binding of tannins with protein.

It has been reported that the polyphenolic content of *Phaseolus vulgaris* alters the biological value (BV) for seeds, both with and without testa. The effect of heat treatment on tannins, however, is not well understood. Decreased levels of tannins after heating are reported but are attributed to different effects such as a decreased extractivity or a change in chemical reactivity (Barroga *et al.*, 1985).

From this section it is clear that the chemical properties of proteinaceous ANF (lectins and protease inhibitors) in common beans make them a good target for heat processing. To study the heat inactivation of these ANF it should be kept in mind that these factors may react differently upon heating whether they are studied as an integral component of the protein or as a purified (crystalline) supplement.

The precise effect of heat on tannins and associated polyphenols, however, remains to be established.

Common procedures for determining the activity of lectins and trypsin inhibitors are given in Table 5. The use of different methods for the measurement of ANF-activities may be the reason for the sometimes contradictory results of investigators for levels in legumes or for residual levels after processing. For example, both protein-type and other-type inhibitors (polyphenols) contribute to the total inhibitory activity towards trypsin. A clear separation between the functional types of inhibitors may help explain differences found between assay procedures.

Table 5. Methods reported for determining (activity of) lectins and trypsin inhibitors.

I.	*Lectins*
	a. Agglutination of animal red blood cells (Valdebouze *et al.*, 1980).
	b. Toxicity to animals (e.g. PER) or other feeding trials.
	c. Specific lectins bands in the protein patterns separated by SDS-Polyacrylamide gel electrophoresis (Koehler *et al.*, 1986).
	d. Enzyme Linked Immuno Sorbent Assay (Hamer *et al.*, 1989).
	e. Functional Lectin Immuno-Assay (Hamer *et al.*, 1989).
II.	*Trypsin inhibitors* (TI)
	a. TI-activity (Kakade *et al.*, 1974; Smith *et al.*, 1980; Van Oort *et al.*, 1989).
	b. TI-units (Hamerstrand *et al.*, 1981).
	c. TI-spottest (Kourteva *et al.*, 1987).
	d. TI-Immunoassay (Brandon *et al.*, 1988).
	e. TI-Affinity chromatography (Roozen and De Groot, 1987).

Furthermore, in relation with *in vivo* assesment of heat treated products, accurate and reliable measurements are required. Therefore, optimization of the assay methods is still a challenge and work is still in progress. Recently, new procedures were developed for determining total and functional lectins (Hamer *et al.*, 1989) and for specific trypsin inhibitors (Roozen and De Groot, 1987).

Methods to remove ANF from beans

To improve the nutritional value of beans and especially the protein from *Phaseolus vulgaris* to their full potential in diets for monogastric animals, removal of ANF-activity resulting in a higher protein digestibility is essential. Several approaches may be considered, including breeding and processing techniques.

Breeding.
Breeding and genetic manipulation of plants are long term efforts in establishing the removal of antinutritional factors while improving the nutritional quality of raw beans. Improving the nutritional value has only recently become a serious consideration in breeding programs (Ma and Bliss, 1978a; Koehler and Burke, 1981). Since bean protein contains up to 12% lectin, a protein which is deficient in methionine, the incorporation of a lectinless allele could improve the nutritional quality of the bean protein if the

Table 6. Processing techniques for common bean processing and principal effects on ANF[1].

Processing	Range for ANF-activity
Fractionation techniques	
Dry milling/air classification	
Protein and starch concentration	
Increase lectins/enzyme inhibitors	171-225% (HA/g)
in the protein fraction	217-235% (TIA/g)
Dehulling	
Decrease polyphenol/tannin content	68- 95% (CE[2] mg/100g)
Increase enzyme inhibitory activity	2- 36% (TIA)
	3- 25% (CTIA)
	27-189% (α-AIA[3])
Increase protein digestibility	2.3-4.3%
Increase protein quality	
Thermal processing	
Wet processes	
Reduction lectin/enzyme inhibitors	up to 100% (HA/TIA)
Reduction essayable levels of polyphenols/tannins	20- 56%
Increase digestibility protein and carbohydrate	
Excess of heat reduces total content and availability of lysine, cystine and methionine	
Dry processes	
HTST-processing: similar to wet processes: some controversial results	
LTLT-processing: decrease nutritional quality.	

[1]Adapted from Bressani & Elias (1988) and compiled from results Elias *et al.* (1979), Elkowicz and Sosulski (1982), Deshpande *et al.* (1982), Rackis *et al.* (1986) and Burns (1987).
[2]CE : Catechin equivalent. [3]AIA: Amylase inhibitor activity.

lectin fraction were to be replaced by a protein fraction containing more methionine (Osborn and Bliss, 1985).

However, in removing antinutritional factors genetically, one has to consider the implications for possible simultaneous consequences in agronomic characteristics like resistance to adverse weather conditions and resistance to diseases and predators. Moreover, bean yield may be affected by changes in ANF (Bond and Smith, 1989). Decreases in ANF-activity of *Phaseolus* beans by breeding has been achieved for lectins (Osborne and Bliss, 1985) and TI (Hymowitz, 1986). Decreasing the level of tannins is also possible for low

tannin genes have been reported to be dominant in common beans (Ma and Bliss, 1978b).

Processing

Many efforts have been made to define the processing conditions, which eliminate or at least strongly reduce ANF-activity in legume seeds. These approaches are largely based on thermal treatment, but other ways have been explored (Table 6).

Heat processing is an effective method for decreasing the activity of protease inhibitors and lectins. The method is based on heat denaturation of these proteinaceous inhibitors. Friedman and co-workers provided experimental evidence for improvement in nutritional value of some legumes, based on alteration of protease inhibitors and lectins by reagents, which disrupt the tertiairy structure of the proteins (disulfide interchange; Friedman and Gumbmann, 1986; Lei *et al.*, 1981). The commercial feasibility of this procedure, however, remains to be evaluated.

Processing, based on the separation into fractions with high and low levels of ANF, is a further possibility. The distribution of ANF in fractionated seeds has been studied for the dehulling process (Deshpande *et al.*, 1982) and for pin-milling and subsequent air-classification. From Table 6 it can be seen that fractionation of feed legumes by dehulling generally increases protein digestibility and protein quality for certain legume species with e.g. high levels of tannins present in the seed coat. This has been indicated for common beans (Deshpande *et al.*, 1982), chickpea (Geervani and Theophilus, 1980) and faba beans (Marquardt, 1989).

Dry milling and separation techniques can be used to produce protein concentrates. Based on the size differential between starch granulas and discrete protein bodies, the technique of air classification can fractionate the legume into its main constituents. This processing technique has been initially studied with respect to ANF (Elkowicz and Sosulski, 1982). These techniques therefore may contribute for each legume to the knowledge of the distribution after fractionation of nutrients and ANF in order to separate into valuable and less valuable products for target animals. The antinutritional effects of these ANF in the protein fraction and the ease of denaturation during thermal processing have still to be evaluated.

Effects of thermal treatments

It has been well established that the nutritive value of vegetable protein is improved by heat treatment. With some exception heat treatment causes an improvement of the nutritive value of legume seeds.

The mechanism through which proteins are better available after heat treatment may result from an increased accessibility of protein to enzymatic attack. Furthermore, and perhaps primarily, this may be due to inactivation of proteinaceous ANF. Protease inhibitors require their structural integrity in order to inactivate proteolytic enzymes by complex formation (Rackis *et al.*, 1986).

The effectiviness of heat treatment on the nutritional value of *Phaseolus vulgaris* depends on a combination of process temperature, heating time, particle size, initial moisture content (dry heat processing) and furthermore the amount of water added during the heat process (wet heat processing). In relation to the temperature/time setting, processing equipment is HTST- (high temperature, short time) or LTLT-designed (low temperature, long time).
Several studies have dealt with trypsin inhibitor (TIA) and lectin activity (the latter measured as haemagglutination activity) (HA) as a function of time and temperature. It has been reported that most of the trypsin inhibitor activity present in the original products is destroyed during normal cooking procedures (Walker and Kochhar, 1982; Rackis *et al.*, 1986). These studies, however, involve, for the greater part, cooking the beans in water. Soaking prior to heating and/or boiling may involve extraction and this reduces the level of water soluble or dispersible compounds in the remaining product. These procedures, therefore, do not simulate actual treatment procedures used for livestock feeding purposes and are not used because they are too costly.
Processing of beans for livestock consumption involves the use of treatments that are more economical. Extrusion, dry roasting, atmospheric steaming (toasting), pressurized steaming (pressure cooking ; autoclaving) have been studied as ways to improve the utilization of beans by industrial and experimental heat processing.

Loss of haemagglutination activity in *Phaseolus vulgaris* by thermal treatment is shown in Table 7.
Proper heat treatment inactivates the level of lectins, based on haemagglutinating activity (HA) in *Phaseolus* beans very effectively, as reported previously (Liener, 1979; Mancini-Filho *et al.*, 1979). Heating *Phaseolus vulgaris* beans at 100°C for at least 20 minutes eliminated the haemagglutination activity (Antunes and Scarbieri, 1980; Reaidi *et al.*, 1981) as well as reduction in performance in rats (toxicity) of beans, according to Grant *et al.* (1982). Lectins from different sources differ in their susceptibility to heat inactivation (Reaidi *et al.*, 1981; Zahnley, 1984): moist heat treatment eliminates lectins from *Phaseolus vulgaris*. Reports on the effect of dry heat, however, are controversial.

Table 7. Effect of thermal treatments on the inactivation (%) of lectin haemagglutinating activity (HA) in common bean (*Phaseolus vulgaris* L.).

Thermal treatment			Bean-type designation	Reduction of HA	References
Proces	Thermal conditions				
	Temp. (°C)	Time[1]			
Autoclaving	100	30	--	82	De Muelenaere, 1964
	121	5	navy bean	100	Kakade and Evans, 1965
	121	30	navy bean	100	Kakade and Evans, 1965
	121	15	small red	100	Myer and Froseth, 1983a
	121	7.5	dry beans	100	Antunes and Sgarbieri, 1980
		15	dry beans	100	Antunes and Sgarbieri, 1980
	121	5	navy beans	99	Yadav and Liener, 1977
		15	navy beans	99	Yadav and Liener, 1977
		30	navy beans	100	Yadav and Liener, 1977
	121	5	kidney bean	74	Tannous and Ullah, 1962
		20	kidney bean	91	Tannous and Ullah, 1962
Steam-heating	100	15	white beans	100	Rodriguez and Bayley, 1987
	100	75	white beans	100	Rodriguez and Bayley, 1987
Extrusion	145	16	small red	98	Myer and Froseth, 1983a
				93	Myer and Froseth, 1983a
	150	16	small red	96	Myer and Froseth, 1983b
Dry roasting	244	108	navy bean	97	Aquilera *et al.*, 1982
	273	133	navy bean	85	Aquilera *et al.*, 1982
	200	23	navy bean	99	Yadav and Liener, 1977

[1]Processing time autoclaving and steam heating in min.; others in sec.

Lectins resist inactivation by dry heat in common beans (De Muelenaere, 1964) or are inactivated by dry heat in winged beans (Kadam and Smithard, 1987). The effect may vary with the nature of lectins (Jaffe and Vega Letta, 1968).

Thermal inactivation of trypsin inhibitor activity (TIA) loss has been investigated intensively and results of these studies show that the extent of destruction of TIA in beans, grits and flours and also in isolated fractions differ widely (Ellenrieder *et al.*, 1980).

TIA in dry beans of *Phaseolus vulgaris* is largely destroyed by heating (Carvalho and Jansen, 1977; Antunes and Sgarbieri, 1980; Sathe and Salunkhe, 1984; Rackis *et al.*, 1986) as can be derived from Table 8. Loss of TIA is almost complete for autoclaving and extrusion processing for certain processing times.

Table 8. Effect of thermal treatments on the reduction (%) of trypsin inhibitor activity (TIA) in common beans (*Phaseolus vulgaris* L.).

Thermal treatment			Bean-type designation	Reduction of TIA	References
Proces	Thermal conditions				
	Temp. (°C)	Time[1]			
Autoclaving	121	30	whole bean	85	Elias *et al.*, 1976
	121	30	meal	29	Elias *et al.*, 1976
	121	5	navy bean	82	Kakade and Evans, 1965
	121	30	navy bean	88	Kakade and Evans, 1965
	120	15	small red	100	Myer and Froseth, 1983a
	121	7.5	dry beans	100	Antunes and Sgarbier, 1980
	121	15	dry beans	100	Antunes and Sgarbier, 1980
	120	8	navy beans	86	Carvalho and Jansen, 1977
	121	5	kidney beans	74	Tannous and Ullah, 1962
	121	20	kidney beans	91	Tannous and Ullah, 1962
	121	5	navy beans	55	Yadav and Liener, 1977
	121	15	navy beans	85	Yadav and Liener, 1977
	121	30	navy beans	100	Yadav and Liener, 1977
Steam-heating	100	15	white beans	65	Rodriguez and Bayley, 1987
	100	75	white beans	97	Rodriguez and Bayley, 1987
Extrusion	145	16	small red	78	Myer and Froseth, 1983a
			small red	98	Myer and Froseth, 1983a
	150	16	small red	100	Myer and Froseth, 1983b
Dry roasting	190	30	navy bean	72	Carvalho and Jansen, 1977
	220	10	navy bean	82	Carvalho and Jansen, 1977
	200	23	navy bean	75	Yadav and Liener, 1977
	273	133	navy bean	54	Aquilera *et al.*, 1982
	244	108	navy bean	77	Aquilera *et al.*, 1982

[1]For steam-heating and autoclaving: time in minutes, other processes in seconds.

The TI of beans, cowpea (*Vigna unguiculata*) and black gram (*Phaseolus mungo*) appears to be resistant to dry heat (Sathe and Salunkhe, 1984). Thermal

stability of TIA in kidney beans has been noted and considered dependent on the concentration of other components present in the protein matrix (Ellenrieder *et al.*, 1980).

Residual TIA after heat treatment indicates that TIA may not be fully eliminated and this residual activity may come (partly) from heat-stable compounds capable of trypsin inhibition (Roozen and de Groot, 1987). Polyphenolic compounds have been shown to account for the majority of the residual TIA in heat treated winged beans (De Lumen and Salamat, 1980; Elias *et al.*, 1979). Some heat resistant components, for example, are residual heat-stable TI, fatty acids and tannins (Tsukamoto *et al.*, 1983b; Padhye and Salunkhe, 1980).

Heat processing is critical in relation to the possible low biological value of proteins. Overheating (in terms of temperature and/or exposure time to heat) may adversely affect the availability of lysine, arginine, methionine and cystine, in intact bean proteins (Rios-Iriarte and Barnes, 1966; Skrede and Krogdahl, 1985). The destruction of amino acids, lysine in particular, upon heating can be estimated in measuring the lysine availability by several assays. Total lysine may be lost on heating but available lysine is depressed even more (Roach *et al.*, 1967). Table 9 shows that available lysine in heat processed common beans is markedly reduced in meals or whole beans, with differences in the degree of loss between bean types.

Table 9. Available lysine value (ALV: g/16 g N) for heat-processed and unprocessed common beans (*Phaseolus vulgaris* L.).

Bean-type designation	Available lysine		Loss of ALV (%)	Assay[1]	References
	Not treated	Auto-claved[2]			
Rosinha G2				A	Antunes and
meal	6.0	3.5	42		Sgarbieri, 1980
albumine	6.3	5.7	10		
globuline	6.5	6.2	5		
White kidney				B, C	Almas and
whole bean	6.3	5.7	10		Bender, 1980
Red kidney				B, C	Almas and
whole bean	6.5	5.1	22		Bender, 1980

[1]Assay procedures: A = Colorimetric method
B = FDNB = Fluoro-dinitrobenzene method
C = DBV = Dye binding method

[2]121°C for 30 minutes.

Overheating proteins may depress digestibility and cause a slower release of amino acids from the protein (De Wet, 1982). Therefore, a safe heating proces is critical to the processing of bean protein in order to establish maximum (protein) nutritional value.

It is clear that thermal treatment of beans has two advantages: it removes the major part of proteinaceous inhibitors at the same time; it may even increase the nutritional value of trypsin inhibitor and protein by making it more accessible. However, each product will have its own thermal optimum. It will be necessary to demonstrate that heat treatment and processing decreases the ANF-activity and will increase in vitro and in vivo digestibility by the same degree.

Kinetic studies

Optimization of heat processing is necessary for an adequate inactivation of trypsin inhibitory and lectin activity. Moreover, the process should result in optimal amino acid availability and in enhaced protein digestibility.
In isothermal process research, systemic thermal analysis is considered an important tool. Varying the temperature and time of heating on feed components and the subsequent determination of the level of ANF in (un)heated samples provide kinetic parameters for the inactivation of ANF.
Studies on the kinetics of the heat inactivation of proteinaceous ANF in relation to processing conditions are reported in soybeans (Labuza, 1973; Cuevas and Cheryan, 1983; Ida *et al.*, 1983), cowpea (Philips *et al.*, 1983) and *Phaseolus vulgaris* (Buera *et al.*, 1984). From these studies Rackis *et al.* (1986) deduced that loss of trypsin inhibitor activity (TIA) was of first order reaction. They established, that the influence of temperature on the reaction velocity can be quantitated in terms of the Arrhenius equation (Charm, 1971).
However, in studying the kinetics of TIA inactivation in soybean suspensions during steam infusion, Johnson *et al.* (1980) found that under all process conditions plots of log [TI], with respect to the time of heating, were curvilinear. This indicated that inactivation in this study did not comform to a single reaction following first-order kinetics and that there may be two separate first-order reactions. The latter reactions are attributed to the inactivation of Kunitz inhibitor (moderate heat labile) and Bowman-Birk inhibitor (more heat stable), respectively (Wolf, 1977; Johnson *et al.*, 1980).
As mentioned before, loss of TIA in *Phaseolus vulgaris* was shown to follow first order reaction kinetics (Buera *et al.*, 1984). They also observed the rate

of TIA loss increased with increasing moisture content (Figure 1). The inactivation energy was found similar to that for loss of available lysine and of nitrogen solubility in heat-treated bean flour.

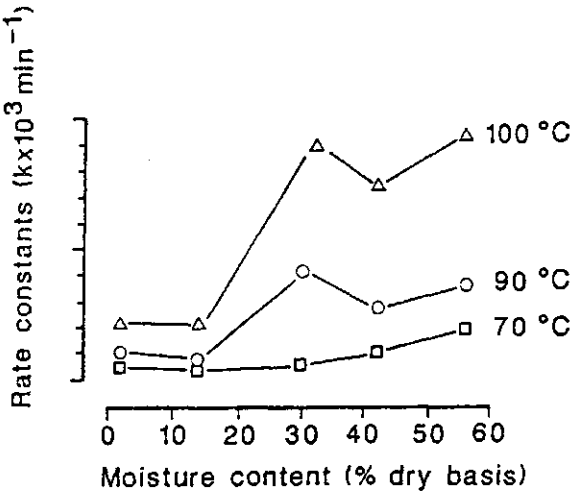

Figure 1. Effect of moisture content on loss in trypsin inhibitory activity in *Phaseolus vulgaris* flours heated at three different temperatures (Buera *et al.*, 1984).

The results of inactivation studies are variable. However, the assays used for TIA and HA measure 'total activity' without discriminating between several molecular forms of the inhibitors. Also, the presence of residual tannins or other substances may account for some TIA remaining after heat treatment (Antunes and Sgarbieri, 1980; Philips *et al.*, 1981, Deshpande & Nielsen, 1987c).

Furthermore it was found that purified preparations of TI in *Phaseolus vulgaris* and soybeans are more heat stable to thermal denaturation as compared with TI in beans or flours (Ellenrieder *et al.*, 1980; Tsukamoto *et al.*, 1983b). In crude extracts of red kidney beans residual TIA was reported to be 10 per cent after heat treatment (60 minutes; 100°C). The purified TI, on the other hand, was heat stable and was only inactivated by heat when high molecular weight (HWM) proteins were present. In this way, combined with heating, HWM substances such as protein and polysaccharides are suggested to be inactivating factors by causing irreversible interactions with the trypsin inhibitor.

Summarizing, the effects of thermal treatment are variable with regard to removal of inhibitory activity. The optimum processing needs to be measured,

therefore, in each bean-type designation. Furthermore we have to question the relevance of measuring only the reduction of ANF after treatment for loss of essential nutrients (Teixeria *et al.*, 1969) is unwanted. Also, the conditions used for any particular heating process have to be described more specifically reporting general processing variables can give misleading interpretation of results.

Effect of processing on protein digestibility

Poor protein digestibility may result in the poor utilization of heat treated seeds of *Phaseolus vulgaris*. The protein digestibility varied between approximately 36 and 56% (raw beans; *in vitro*) and between 62 and 78% (*in vivo*; rats) (Bressani and Elias, 1978). The reasons for this low digestibility are not well understood. These are probably due to a combination of factors. Also, comparison of the results of digestibility studies are not possible because of the use of different heat treatments. The varying circumstances, under which biological assays and *in vitro* studies are carried out, also contribute to controversial results.

In vitro proteolysis of isolated protein fractions

Numerous attempts have been made to explain the poor protein digestibility of *Phaseolus vulgaris* on the basis of *in vitro* digestion experiments involving the action of various proteolytic enzymes on the major storage protein. The latter, comprising about half of the total protein in the mature seed (Ma and Bliss, 1978a; Deshpande and Nielsen, 1987b), has been variously referred to as phaseolin, globuline, glycoprotein II, G1, or simply as the salt-soluble fraction, as pointed out before. These *in vitro* studies have revealed that the native protein is quite resistant to digestion by purified proteases, singly or in combination, whereas the protein which has been denatured by heat is readily hydrolysed under the same conditions (Seidl *et al.*, 1969; Romero and Ryan, 1978; Liener and Thompson, 1980; Philips *et al.*, 1981; Marquez and Lajolo, 1981; Deshpande *et al.*, 1983; Deshpande and Nielsen , 1987a, 1987c). The resistance of unheated phaseolin to enzymatic attack has been attributed to the compact, three-dimensional structure of the native protein which becomes unfolded by heat denaturation, thus exposing vulnerable peptide bonds. Since phaseolin is a glycoprotein (Chang and Satterlee, 1981; Deshpande and Nielsen, 1987b), the possibility that bound carbohydrate might also impose a steric barrier to proteolysis has also been proposed (Semino *et al.*, 1985).

Sgarbieri *et al.*, 1982, however, have questioned the relevance of the results obtained by *in vitro* experiments involving the use of pure endopeptidases,

since they found that even undenatured phaseolin was readily digested by sequential treatment with an extract of the rat stomach followed by intestinal extracts. Heat treatment (100°C for 10 min.) appeared to make little difference in this regard. *In vivo* studies with rats by the same author confirmed the ready digestibility of the native protein (80%- 90%), although, in this case, there was an increased production of endogenous protein which was lost to the animal. When this latter effect is taken into account, the apparent digestibility of phaseolin is only about 35%.

Compared with phaseolin, few studies have dealt with the digestibility of the albumin fraction which constitutes about 40% of the total protein (Deshpande and Nielsen, 1987b). Unlike phaseolin, whose resistance to proteolysis is abolished by heat denaturation, the albumin or water-soluble protein fraction of *Phaseolus vulgaris* becomes less digestible following heat treatment (Marquez and Lajolo, 1981; Deshpande and Nielsen, 1987c). The reason for this difference in the manner whereby phaseolin and albumin are affected by heat treatment is not clear, but possible explanations include heat-induced interactions with tannins (Aw and Swanson, 1985) or protein-protein interactions resulting in the formations of indigestible protein aggregates stabilized by disulfide linkage (Deshpande and Nielsen, 1987c). Also, the possible role that heat-stable trypsin inhibitors, shown to be present in the albumin fraction (Marquez and Lajolo, 1981; Deshpande and Nielsen, 1987c), may play in these experiments needs to be elucidated. In any event, the relevance of the few *in vitro* experiments which have been done with the albumin fractions remains to be established *in vivo*.

Protein digestibility in vivo

Heat processing improves the digestibility of legume proteins by destruction of TI/lectins and by opening of the protein structure through denaturation. Nevertheless, even when antinutritonal factors are no longer biologically active, digestibility of the protein in *Phaseolus vulgaris* is less than for most vegetable proteins and significantly less than for animal protein.

In Table 10 the *in vivo* apparent digestibility coefficient and N-retention for heat processed bean in pigs are given. Faecal apparent digestibility coefficients, especially for protein are rather low as compared to soybeans or peas (Van der Poel, 1989). Nitrogen faecal digestibility ranged from 63 to 79% with the exception of the data of Rodriguez and Bayley (1987) and the short time (20 minutes) processing of beans reported by Van der Poel *et al.* (1989). Of course, for correct comparison, the inclusion levels in the diet and the age of the piglets have to be taken into account.

It can be expected that protein digested in the ileum will be a better predictor of potential nitrogen use because absorbed nitrogen from the hindgut is not in

the form of amino acids (Pond, 1987). Indeed, the amount of digestible crude protein and amino acids, measured at the terminal ileum, has a higher

Table 10. *In vivo* digestibility (%) and N-retention (N-ret., g/day) for heat processed[1] (*Phaseolus vulgaris*) beans in pigs.

Treatment	Digestibility				N-Ret.	References
	Faecal		Ileal			
	N	TS[2]	Lys.	N		
Raw beans	neg.					Van der Poel *et al.*, 1989
Autoclaved 121°C; 15 min.	68	70	71		6.3	Myer and Froseth, 1983a
Extruded 150°C; 16 sec.	79	74	80		7.6	Myer and Froseth, 1983a
Steam-heated for 75 min.	43					Rodriguez and Bayley,
Steam-heated						Van der Poel and Huisman[3], 1988
for 20 min.	43.7			-40		Van der Poel *et al.*[4], 1989
for 40 min.	67.1			10		
for 80 min.	67.8			40		
Steam-heated[5]	63			53		Huisman and Van Weerden, 1987
Heated[6]	74					Smits, 1989

[1]100°C [2]Total sulphur [3]Ileal data [4]Faecal data [5]Commercially sample [6]Mean of digestibility coefficients of heat treatments under various conditions (B. Smits, personal communication, 1989).

correlation to the protein deposited in pigs, as recently reported by Dierick *et al.* (1988). From Table 10 it can be seen that ileal apparent digestibility coefficients of nitrogen differentiate more with prolonged heating times, but still are very low. The reasons for this poor digestibility are not well defined and a shorter transit time in the digestive tract after feeding and residual activity of ANF after processing are cited to be at least partially responsible (Bressani and Elias, 1988). The extent of endogenous protein in the establishment of apparent nitrogen digestibility has to be measured.

Discussion

The nutritive value of protein in beans is related to the presence of

antinutritional factors (ANF) and the inherent proteolytic resistance of the storage proteins. The effects of ANF *in vivo* are:

1. damage to the gut wall brush border resulting in interference with normal intestinal digestion and/or absorption of nitrogen and tissue protein catabolism (lectins)
2. inactivation of trypsin and other enzymes (both specific and non-specific) with consequences for apparent protein digestibility
3. protein complexing by tannins, related polyphenols and phytate (both enzymes and bean proteins)
4. increase of endogenous nitrogen output.

Changes in protein quality with processing will be critical to the final nutritional quality of the treated products. For the nutritional significance of the negative effects of the ANF, it is important that *in vivo* measurements help establish threshold levels, at which these ANF may not exert adverse effects. We need effective processing methods to produce safe and high quality beans as an economical ingredient in diets for monogastric animals. It should however be emphasized, that animal species respond differently to similar diets (Struthers and MacDonald, 1985). This species difference is also found with diets containing common beans (Huisman and Van der Poel, 1989).

Separate effects of the various antinutritional factors on nutritional value are not well established. The nutritional significance of a specific ANF is often obscured by the simultaneous presence of other ANF. Absence of the major effects other than trypsin inhibitory activity suggest that the improvement in nutritional value of *Phaseolus mungo* can be sought through inactivation of the TI in it (Padhye and Salunkhe, 1980).

Studies with raw *Phaseolus* beans indicate a more pronounced role of lectins rather than TI or polyphenols (Liener and Kakade, 1980; Philips *et al.*, 1981; Grant *et al.*, 1983). These lectins are responsible for impaired growth and even death in animals (Koehler *et al.*, 1986).

Antinutritional factors as well as the resistance of bean protein to proteolysis affect digestibility and absorption and, consequently, protein nutritional value. These values are estimated by *in vivo* measurements of protein quality like rat growth studies (PER: protein efficiency ratio; NPU: net protein ratio). Other measurements used are biological (Tetrahymena) or chemical/enzymatical (*in vitro* estimate of digestibility) assays, respectively. The values obtained from rat assays are considered estimates for protein nutritional value in general. Their significance in terms of protein nutritional value for humans is not well defined (Bodwell, 1977) and *in vivo* growth trials indicate a clearly different response for rats and swine to diets with legume proteins (Huisman and Van der Poel, 1989).

The lectins in raw and moderate heat-treated *Phaseolus vulgaris* proteins have extreme negative effects on brush border epithelial cells (Pusztai, 1987; Kik *et al.*, 1989). These effects are reported for rats (Pusztai *et al.*, 1979; Pusztai *et al.*, 1981) and for pigs (Van der Poel and Huisman, 1988). The direct effects of the lectins may thus override the importance of any additional nutritional deficiencies of other *Phaseolus* bean proteins or ANF (Sgarbieri *et al.*, 1982) in digestibility studies. Consequently, digestibility coefficients for raw and inadequately processed bean protein derived from animal studies, will be low. Experiments carried out with either isolated intestinal preparations, isolated bean protein fractions or reactions with (multi)enzyme techniques do not reflect the true actual physiological state of the intestine and, consequently, the actual absorption by the animal (Donatucci *et al.*, 1987). These measurements, therefore, cannot serve as a unique criterion for protein nutritional value of unprocessed or inadequately processed *Phaseolus* bean proteins. For adequate processed bean proteins of which denatured bean lectins are no longer capable of binding to the mucosa cell membrane, these protein quality measurements may be more valid as criteria for the degree of inactivation of lectin activity by processing.

Many experiments aimed at optimizing processing conditions, especially the setting of time/temperature relation in achieving low levels of residual inhibitory activity, have been reported. From these studies a general agreement has evolved that the inactivation of ANF, proteinase inhibitors and lectins in particular, can be achieved by heat treatments. This phenomenon is based on heat denaturation of these proteinaceous ANF. It should hoewever be emphasized, that TIA determined by some chemical assays, may partly result from polyphenolic compounds and even from fatty acids present in samples. Trypsin inhibitor activity and residual TIA after heat processing e.g. is attributed to inhibitory activity, other than protein-type inhibitors (Roozen and De Groot, 1987). Polyphenols, e.g., are known to be less heat-sensitive. Studies on factors for the optimizing of nutritional value of *Phaseolus vulgaris*, are rare (Antunes and Sgarbieri, 1980).

The extent of improvement on protein digestibility by heat processing depends on treatment procedures. Moderate heating is often necessary for improving nutritional quality but severe heating, attributed to applied heat or induced heat by auto-oxydation, is indicated to result in harmfull effects (Roach *et al.*, 1967). Excessive heating may cause essential amino acids to decompose and/or to react chemically with other compounds which affects amino acid availability.

So far, only little attention has been given to the biological availability of essential amino acids of bean proteins in relation with the conditions of storage (methionine) and of processing (lysine; S-containing amino acids) (Molina *et*

al., 1975, 1976). It is not known whether HTST- or LTLT-processing cause less reduction in amino acid availability.
For inactivation of proteinaceous ANF, hydrothermal processing seems to be more effective compared with dry heat applications. The integral structure of the proteins is affected intensively in the presence of moisture, and thus facilitates proteolysis. Complete inactivation of these heat-sensitive factors, however, is not possible (Friedman and Gumbmann, 1986; Grant *et al.*, 1983). Presumably, it is due to the heat resistance of toxic lectins (Jaffe, 1980) and some forms of trypsin inhibitors (Padhye and Salunkhe, 1981). There seems to be only little correlation between residual activity of TIA and lectins and *in vivo* nutritive value. It is known that heat stabilities of the various proteinaceous ANF differ markedly between varieties. In this regard it is noticable that inactivation of proteinaceous ANF by heat denaturation may be reversible.
Removal of approximately 90% or more of protease inhibitor activity is sufficient to abolish the negative effects for rats. For pig nutrition, however, this has not been elucidated. Maximum protein efficiency ratio (PER) in rats e.g. was observed when considerable residual TIA and HA were still present (Antunes and Sgarbieri, 1980). This was explained by the fact that some very active or toxic forms of TI or HA are extremely heat labile thus being destroyed very rapidly by treatment. These results point to the need for functional lectin and TI assays, respectively. These assays should differentiate between different types of the ANF establishing a detailed analysis of the various biologically active constituents.
Subsequently, the heat inactivation patterns as well as the relationship to *in vivo* effects with target animals, need to be established.

It seems interesting to study the kinetics of the thermal treatment of beans for its contribution in optimizing the inactivation of ANF while providing minimal degradation of essential nutrients. Contrary to soya beans, there have been only a few studies involving proces optimization in relation to ANF-inactivation in *Phaseolus vulgaris* beans.
Buera *et al.* (1984) studied the kinetics of TIA loss in heated beans and reported that the dependance of the activation energy for TIA loss on moisture content was similar to that found for the loss of available lysine and nitrogen solubility, respectively.
These studies also point to the need for reliable and rapid functional analytical assays in a way rapid screening methods are available for obtaining kinetic data. In addition to isothermic process studies, normally used for thermal process optimization, the use of Differential Scanning Calorimetry (DSC) as a potential means for pre-sreening process variables has been proposed (P.J.

van der Steen, personal communications, 1989; Vooijs *et al.*, 1989).
In optimizing the protein and/or energy value of heat processed beans, *in vivo* evaluation of beans will be more valid than residual levels of ANF. This means that the chemical analysis need to be calibrated with *in vivo* experiments.
A precise control of processing conditions must be implemented, apart from reliable quality control parameters. Moreover, inactivation procedures must include, at least, short-term animal feeding experiments to relate nutritional parameters and ANF inactivation data.

Feeding of bean-containing diets to animals often results in extra loss of nitrogen not only in the faeces but also in the urine. The high loss in the faeces may result from either a low digestibility per se or because ANF in beans cause an increased secretion of endogenous proteins. Therefore, apparent faecal digestibility of protein from *Phaseolus vulgaris*, especially for inadequately processed beans, is low. The extra loss in the urine may result from microbial degradation in the large intestine. The resulting ammonia is absorbed, converted to urea and excreted in the urine.
It has already been discussed that swine ileal digestibility gives a more reliable estimate of nutritional protein value (Dierick *et al.*, 1988).
The increase in nitrogen output may be attributed to the low level of absorbed amino acids from bean protein and thus come directly from the feed consumed. Lectin activity is known to interfere with absorption by disorganising the brush border cell membrane and increase the permeability of the small intestine for macromolecules and lectins with consequent effects on systemic metabolism and on the immune system (Pusztai, 1987). Feed digestion and nutrient absorption will therefore be reduced and cellular protein and mucin synthesis and secretion are increased.

In conclusion, studies with *Phaseolus vulgaris* indicate a more pronounced role for lectins, rather than trypsin inhibitors and polyphenols. The relationships among heat processing of beans and final protein nutritional value in target animals will require more detailed information regarding the residual ANF-activities upon based on functional assays after processing.
Furthermore, study is needed on the patterns of *in vivo* protein digestibility to result in increased understanding of nitrogen partitioning in feed or endogenous origin.
These studies will contribute to a more complete understanding in optimizing the conditions of heating to derive maximum nutritional advantage from thermal treatments of *Phaseolus vulgaris* beans for livestock feeding.

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CHAPTER II

Air classification of Bean Flour - Effects on Protein, Antinutritional Factors and the Effect of a Fines Fraction on Cultured Explants of Small Intestinal Mucosa.

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Abstract

Common beans (*Phaseolus vulgaris* L., *cv Processor*) were ground in different mills followed by air classification at two different classifier settings. These procedures yielded fines fractions containing protein levels ranging from 40.0% to 52.6%, depending mainly on cut points with classification. In the fines fractions, the protein content was at least double the level of the initial flour. Furthermore, levels of trypsin inhibitor activity and total and functional lectins in the fines fractions were one to four-fold those in the initial flour. The coarse fractions, however, were not free of these antinutritional factors. Villus length in cultured explants of small intestinal mucosa was decreased in the presence of an air-classified fines fraction. In addition, microvillus vesicles were formed.

From the present experiment it is concluded that air classification is effective in producing protein concentrates from *Phaseolus* beans. A clear separation of constituents with lectin or trypsin inhibitory activity was not established. Based on the pathological effects of its constituents, fines fractions cannot be used in feeding practice without prior elimination of the lectins.

Introduction

Dry beans (*Phaseolus vulgaris* L.) contain several factors which interfere with digestion when ingested in the raw state by animals and humans. These factors are referred to as antinutritional factors (ANF) and, for common beans, lectins (Liener, 1986; Pusztai, 1987; Kik *et al.*, 1989) and protease inhibitors (Sgarbieri and Whitaker, 1982) have been shown to exert negative effects. In the literature, therefore, special attention has been paid to the effect of lectins on the extent of feed digestion and intestinal disturbance (Pusztai, 1987; Kik *et al.*, 1989).

Heat processing is an effective method to decrease the activity of lectins and protease inhibitors (Van der Poel, 1990). Separation of legumes into fractions with high and low levels of ANF may be a further possibility. Fine milling followed by fractionation of the flour by air classification has been shown to produce protein and starch concentrates from some grain legumes (Kon *et al.*, 1977; Sosulski and Youngs, 1979; Tyler *et al.*, 1981). The fineness of grinding and/or the cut point employed for classification of legume flours, however, have been observed to affect the composition of constituent proteins (Patel *et al.*, 1980; Reichert and Youngs, 1978; Sosulski *et al.*, 1987). Furthermore, there is little published data on the distribution of antinutritional factors in fractions after milling and air classification of legumes; the work of Elkowicz and

Sosulski (1982) being notable in this respect.
The objective of the present investigation was firstly to produce a protein concentrate from beans through air classification. The effect of type of milling and changing cut point on the fractionation of *Phaseolus* bean flour was also examined as was the impact of dry fractionation on the distribution of total and functional lectins as well as trypsin inhibitory activities. Finally, a fines fraction was used to study its effect on the morphology of cultured explants of pig small intestinal mucosa.

Materials and Methods

Seeds of common beans (*Phaseolus vulgaris* L., *cv. Processor*) were obtained from a commercial supplier.

Processing

Milling procedures for the preparation of whole bean flour were carried out using a Pallmann mill (at 5.000 rev min^{-1}) or an Alpine 160Z pin mill (at either 11.000 or 14.000 rev min^{-1}), respectively. Prior to pin-milling procedures, beans were hammer milled (Condux LHM 20/16) to pass a 4-mm screen. Beans were not conditioned to a lower moisture content or dehulled prior to milling.
Air classification was carried out on an Alpine laboratory Zig-Zag 100 MZR classifier. After initial milling and classification of the whole flour (WF), a fines (FI) and coarse (CI) fraction were obtained. The bean coarse fraction (CI) was successively milled and passed through the classifier. This procedure resulted in a second fines fraction (FII) and a final coarse fraction (CII) as described by Aguilera *et al.* (1982). The weights of the initial fraction prior to air classification and of the coarse fraction after classification were recorded, the difference being taken as the weight of the fines fractions.
Classification of 500 g lots of bean flour was investigated at cut points of 10 μm and 20 μm. Cut points were changed by varying the air flow rate settings and classifier speed. These variables were derived from preliminary experiments with limestone and mass density measurements of the beans used. Classifying speed values of 8700 and 5400 rev min^{-1} were used with corresponding air flow rates of 46.3 and 49.6 $m^3 h^{-1}$, respectively (Table 1).

Table 1. Calculated and defined cut points used for air classification

Classifier setting		Cut-point (μm)	
Speed rev min^{-1}	Air flow rate $m^3 h^{-1}$	Calculated	Measured[1]
8700	46.3	10	11.8 ± 1.71
5400	49.6	20	18.0 ± 1.41.

[1]Mean ± SD (n=5); derived from particle size analysis, using method 2 of Wright *et al.* (1984).

The established cut points for fractionation were confirmed by particle size measurements and were defined by the method of Wright *et al.* (1984). The cut point is defined as the particle size at which the yield of undersize particles in the coarse fraction is equalled by the yield of oversize particles in the fines fraction.

Particle size distribution of fractions and flours were determined using a Malvern 3300 P Particle Sizer equipped with a 100 mm lens and using procedures outlined in the manufacturer's manual. The procedure is based on the principle that light, produced by a low power visible laser transmitter, is diffracted by the particles illuminated to give a stationary diffraction pattern. By integration over a suitable period and using a continuous flux of particles, a representative bulk sample will be obtained.

All particle size measurements were carried out in quadruplicate on samples dispersed in propan-2-ol. Two sets of five different air-classified fractions of beans and peas were used to plot particle size curves and to derive the cut point at a given classifier setting.

Analytical methods

The samples were analysed in duplicate for their dry matter and nitrogen content following standard methods (AOAC, 1975) in samples of fines and coarse fractions and in WF. For crude protein, a nitrogen-to-protein conversion factor of 6.25 was used throughout. Duplicate analysis of trypsin inhibitor activity were carried out on WF and resulting fractions according to a modified Kakade assay (Kakade *et al.*, 1974) as described by Van Oort *et al.*

(1989).
Analysis of total lectins was carried out on whole flour and all fractions using the haemagglutination method. Haemagglutination activity (HA) was measured using rabbit red blood cells according to the method of Valdebouze *et al.* (1980). In addition, for Pallmann-milled WF and its resulting fractions, total as well as functional lectin contents were measured. The ELISA-method (Hamer *et al.*, 1989) using a coating of rabbit anti-*Phaseolus* lectin antibodies, was used for determining total lectin contents in quadruplicate samples. Analysis for functional lectins was performed in quadruplicate by a functional lectin immuno-assay (FLIA-BBM) using a coating of porcine small intestinal brush border membrane (BBM) as described by Hamer *et al.* (1989). ELISA and FLIA units are expressed relative to lectin levels (58 mg/g and 52 mg/g, respectively; 100%) of the whole flour.
Total polyphenols were determined using the method described by Swain and Hillis (1959).

Cultured explants of pig small intestinal mucosa

Segments of small intestinal mucosa from specific pathogen-free piglets (7 weeks old), were cut open longitudinally and washed in Trowell's T-8 medium. The mucosa was separated and small explants (9 mm^2) were cut and placed on triangular cut stainless steel grids in sterile plastic organ-culture dishes (Danielsen *et al.*, 1982). To the central well of the dish 0.9 ml Trowell's T-8 medium was added for the control explants, or different concentrations (10, 20, 40, 80, 160 and 320 μg/ml in T-8 medium) of the air classified FII-fraction (Pallmann milled; cut point 10 μm for the test samples). The outer ring was filled with 3 ml double distilled water. The dishes were covered, put in an incubator at 37 °C and gassed for 15 minutes with carbogenic air. Explants cultured for 5 h were taken for light- and electron microscopical examinations.
For morphometrical analysis, one explant from each dish was fixed in 4% buffered formalin solution and embedded in paraffin. Serial sections (5 μm) were cut and stained with haematoxylin and eosin. The length of 10 well-orientated villi (v) and crypts (c) were measured at 100 x magnification by means of a TEA Image Manager (TIM, Breda, The Netherlands). For transmission electron microscopy, explants were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer. Ultrathin sections were stained with uranyl magnesium acetate and lead citrate and examined with a Philips EM410LS electron microscope at 60 kv.

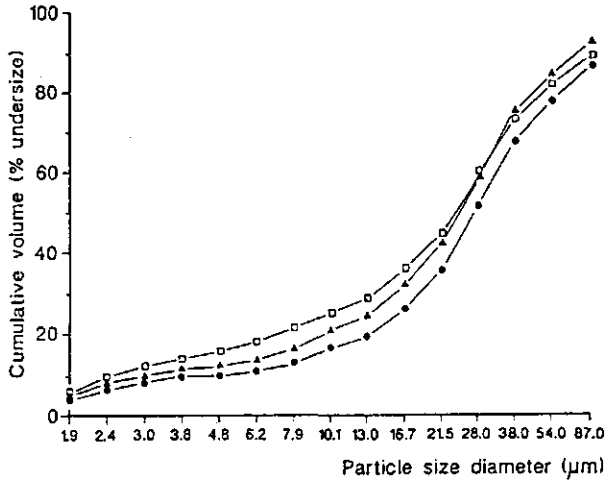

Figure 1. Particle size distribution of bean flour obtained after a single pass through the various mills. □ , Pallmann (5000 rev min^{-1}); ▲ , Pin mill (14000 rev min^{-1}); ● , Pin mill (11000 rev min^{-1}).

Results

Fine milling

The milling efficiency was investigated by particle size distribution of WF obtained by different mills and milling procedures, respectively. The results of particle size analysis (PSA) of WF investigated are shown in Figure 1. Whole bean flour showed no large differences in particle size distribution due to milling procedures. Pallmann-milled flour showed the greatest percent particles undersize at specific particle diameters up to 28 μm. More than 60% of the particles have diameters less than 28 μm which is approximately 5% more in comparison with particle size of flour, pin-milled at 11.000 rev min^{-1}. Particle size values of pin-milled flour at 14.000 rev min^{-1} are intermediate. The initial temperatures of whole flour (19.5 °C) and coarse fractions (21.1 °C) increased only slightly by milling and mean temperatures measured after milling were 30.1 and 24.1 °C, respectively.

Air classification

As a preliminary experiment, the reproducibility of the Alpine A 100 MZR was investigated by air classification (5400 rev min^{-1}; air flow of 49.6 m^3 h^{-1})

of samples of whole bean flour in quadruplicate. Recovery of the protein content of the fines and coarse fractions was determined and protein levels of these fractions were 44.5% ± 0.12 and 13.8% ± 0.27, respectively. For the classification studies, actual cut points were calculated from particle size measurements of five batches of pea and bean after milling and classification. The established cut points for fractionation at the previously described classifier speeds and air flow rates were approximately 11.8 μm and 18.0 μm, respectively (Table 1). In addition, Figure 2 illustrates the particle size distribution of the two fines (FI, FII) and the coarse (CII) fractions at two different classifier settings. This figure illustrates the effect of air classification on the separation of both fine and coarse particles from Pallmann-milled flours. FII fractions have a greater percent particles undersize as compared to FI-fractions at almost all particle diameters. For the CII-fraction some 80% of the particles exceed a diameter of approximately 20 μm. At lower classifier settings, the mean particle size in the fines fractions is less than that observed at higher settings.

Yield and composition

Dry weight losses during classification were corrected by obtaining the yield of the fines fractions by subtracting the weight of the coarse fraction from the initial flour weight.

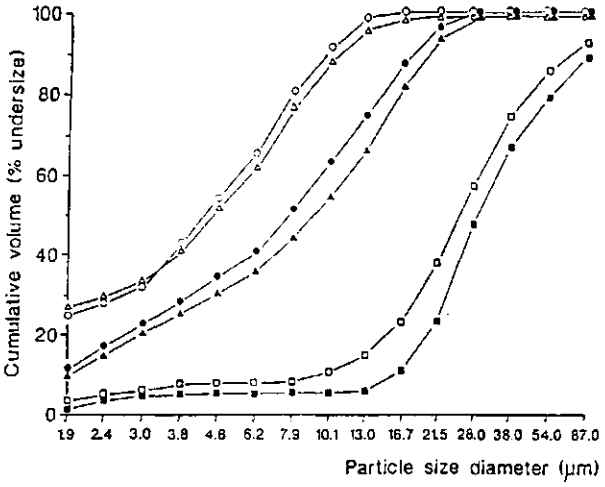

Figure 2. Particle size distribution of the fines fractions and the coarse fraction of bean flour after dry fractionation at two classifier settings: ○ , FI 10 μm/●, FI 20 μm; △ , FII 10 μm/▲, FII 20 μm/; □ , CII 10 μm/■ , CII 20 μm.

Table 2. Yield (%), protein level (CP; Nx6.25) and dry matter content (DM; %) of Pallmann-milled bean flour and of fines and coarse fractions after air classification.

	Cutpoint					
	10 μm			20 μm		
	Yield (%)	DM (%)	CP (% DM)	Yield (%)	DM (%)	CP (% DM)
Whole flour	100	89.8	21.4	100	89.9	21.4
Fractions						
FI	12.4	91.7	51.3	17.9	91.5	47.7
CI	87.6	--	--	82.1	--	--
FII	5.5	91.4	48.1	6.6	91.4	42.6
CII	82.1	90.3	15.6	75.5	89.8	12.6
Recovery CP			102			98

In Table 2 yield and analytical data are given on Pallmann-milled fractions, air classified at two different cut points. The moisture content of whole bean flour was approximately 10%. For the fines fractions the dry matter content was somewhat higher as compared to the initial flour and the coarse fraction. The protein levels recorded in the fines fractions (FI and FII) of beans at the cut point of 10 μm were 51.3% and 48.1%, respectively. From Table 2 it can be calculated that these protein values imply a protein separation into the combined fines fractions of 42% and 53% of the total bean protein content at cut points of 10 and 20 μm, respectively.

Yield, dry matter and crude protein content of flour derived from two pin-milling procedures and those of resulting fractions are summarized in Table 3. Fines fractions FI and FII, with yields of 7.6% (52.6% protein level) and 3.4 (48.3% protein level), respectively, were obtained at pin-milling operations of 11.000 rev min^{-1} and a fractionation cut point of 10 μm.

Increasing the classifying speed or pin-milling at 14.000 rev min^{-1} results in higher yields of both fines fractions. Crude protein levels in FI and in FII are not greatly affected by milling procedures. Classifier settings affect the level of protein and the protein level of FI was observed to be approximately 5% higher than that of FII. Moisture contents of the whole flour and equivalent fractions after air classification showed similar patterns for both milling and classifying procedures (Tables 2 and 3). Recovery of protein in FI, FII and CII fractions, respectively, as a percentage of the initial WF protein, ranged from 97 to 102% .

Table 3. Analytical data[1] on air classified fractions of Phaseolus beans obtained by different type of pin-milling at two cut points.

		Whole flour	Fractions				Recovery CP
			FI	CI	FII	CII	
Pin milling							
11.000 rev min^{-1}							
10 μm	Yld	100	7.6	92.4	3.4	89.0	
	DM	88.4	91.4	--	91.0	90.0	
	CP	20.4	52.6	--	48.3	16.1	98
20 μm	Yld	100	13.3	86.7	6.1	80.6	
	DM	88.4	90.9	--	90.8	88.8	
	CP	20.4	47.0	--	43.3	14.1	99
14.000 rev min^{-1}							
10 μm	Yld	100	10.5	89.5	3.9	85.6	
	DM	89.2	91.4	--	91.3	89.5	
	CP	20.3	52.1	--	47.0	14.7	98
20 μm	Yld	100	16.7	83.3	7.0	76.3	
	DM	89.2	91.1	--	91.3	89.5	
	CP	20.3	48.1	--	40.0	11.6	97

[1]Yld, Yield; CP, crude protein in DM; DM, Dry matter

Antinutritional factors

Total polyphenol contents of WF flour and of all fractions after dry fractionation were relatively low and ranged from 0.35-0.36% in the WF. Values were highest in the fines fractions (range: 0.61-0.72%) and were lowest in the coarse fraction (range: 0.18-0.31%). In view of these relatively low levels, data on polyphenols contents are not included in Tables 4 and 5.
The relative distributions of proteinaceous ANF between the fines and coarse fractions are shown in Table 4. The levels of TIA and lectin activity (HA) in WF differed slightly between the flours used for pin-milling and Pallmann-milling procedures, respectively. Beans were used from the same batch and pin-milling was carried out after only a two weeks storage of the whole beans at 4°C.

Table 4. Distribution of total lectins (HA)[1] and trypsin inhibitor activity (TIA)[2] in whole flours and fines and coarse fractions.

	HA				TIA			
		Flour	Fractions			Flour	Fractions	
		FI	FII	CII		FI	FII	CII
Pallmann mill								
5000; 10 μm	100	320	320	80	6.46	7.89	16.65	3.52
5000; 20 μm	100	320	320	80	6.46	23.20	7.84	2.39
Pin mill								
11000; 10 μm	80	160	320	64	4.46	6.47	4.43	6.01
11000; 20 μm	80	128	320	80	4.46	8.78	11.84	3.12
Pin mill								
14000; 10 μm	64	160	160	100	4.46	5.77	14.69	3.18
14000; 20 μm	64	128	200	50	4.46	8.41	20.03	2.49

[1]Units per g of sample
[2]Mg trypsin inhibited/g of sample

Data in Table 4 indicate that TIA and HA are concentrated in the fines fractions during air classification. The coarse fractions, in general, contain lower levels of TIA and HA (Table 4) compared to the flour.

For TIA, milling procedures prior to fractionation affect the distribution between FI and FII. The CII fraction of air classified pin-milled flour showed the highest TIA levels while for Pallmann-milled flour a four-fold enrichment was determined in the FI fraction.

For total lectins, measured by haemagglutination activity, the relative distribution showed a more regular pattern with activities concentrating with the fines fractions (Table 4). The highest activity was determined in the fines fraction after re-milling (FII). It is notable that HA levels in CII are very similar to the level in the initial flours, indicating that the coarse fraction was

not free of HA. Data on the lectin content measured by more sensitive procedures for total and functional contents are presented in Table 5. Total lectin activity as determined by different assays (ELISA; FLIA) showed similar distribution patterns for the fines and coarse fractions. In general, lectins (ELISA) separated to a large extent with the fines at the classifier settings investigated. The lectin content assayed with ELISA, however, showed different levels for the two fines fractions, contrary to the HA assay. In addition, these data indicate a lower absolute lectin content in both the fines and coarse fractions as compared to contents measured by the haemagglutination assay. Functional lectins (FLIA), however, separated with the fines to a larger extent as compared with ELISA total lectins. Classification of flour at a cut point of 10 μm results in two fines fractions with equivalent functional lectin content; classification at a higher cut point show no large differences for the FI fraction but indicate the FII fraction to have a lower content of (functional) lectins as measured by both ELISA and FLIA.

Table 5. Distribution of total (HA; ELISA) and functional lectins (FLIA-BBM) in Pallmann-milled *Phaseolus* beans and air classified fractions[1].

	10 μm			20 μm		
	Total		FLIA (%)	Total		FLIA (%)
	HA units/g	ELISA (%)		HA units/g	ELISA (%)	
Whole flour	100	100	100	100	100	100
Fractions						
FI	320	226	312	320	243	305
FII	320	228	295	320	180	202
CII	80	55	59	80	52	49

[1]For explanation of assay abbreviation, see text.

Cultured mucosal explants

The results of the morphometrical analysis are summarized in Table 6. In explants cultured in the presence of higher concentrations of the air-classified fraction, the v/c ratio was decreased due to a decrease in villus length.

Table 6. Morphometric variables of jejunal explants (cultured for 5 h) in the presence of an air classified fines fraction (ACF) of *Phaseolus* bean.

ACF level μg	Villus length % + SEM	Crypt depth % + SEM	Ratio (v/c) %
0	100.0 ± 5.1	100.0 ± 5.1	100
10	96.6 ± 5.8	107.6 ± 4.4	88.8
20	92.2 ± 6.6	99.4 ± 7.0	94.4
40	96.4 ± 5.9	107.2 ± 6.0	88.8
80	80.0 ± 4.2	103.2 ± 4.0	75.2
160	85.3 ± 3.5	95.8 ± 2.7	81.1
320	81.1 ± 3.6	103.2 ± 2.8	81.1

Furthermore, at the ultrastructural level, higher numbers of microvillus vesicles than normal had formed (Figures 3 and 4). The microvilli were shortened and irregular positioned.

Figure 3. Ultrastructural picture (33.570 x) of cultured villus epithelium (control).

Figure 4. Ultrastructural picture (46.350 x) of villus epithelium cultured in the presence of 160 μg/ml air classified *Phaseolus* bean fraction. Note the presence of microvillus vesicles.

Discussion

Based on the different size and density of discrete protein bodies and starch granules, the technique of air classification after fine milling fractionates legumes into their main constituents, protein and starch. The results of the present studies show that fine milling and subsequent air classification may be used to produce bean protein concentrates. Regrinding and air classification of the coarse fraction (CI), initially introduced by Vose *et al.* (1976), results in further enrichment of protein from the starch in the fines fractions.

The yield and composition of the different fractions produced by air classification are primarily fixed by the relative proportions of protein and starch in the grain legume seeds under investigation. Furthermore, the yields of fractions are affected by the initial moisture and oil contents of the seed prior to processing (Aguilera *et al.*, 1982; Reichert, 1982). The importance of initial size reduction prior to classification has been demonstrated previously (Wright *et al.*, 1984).

The fat content of the *Phaseolus* beans used was very low (1.2%), therefore, fat is not expected to play an important role in the efficiency of the air classification process.

The yields of fines fractions in this study were lower than the yields of Northern or Navy bean fractions using similar fractionation procedures but different processing conditions. The beans used in this experiment had a moisture content ranging from 10.1 - 11.6 percent (Tables 2 and 3). These levels are higher than reported in the literature and they may have been suboptimal for ideal classifying conditions. Higher yields were obtained in previous air classifying studies with common beans by Tyler *et al.* (1981) and Sosulski *et al.* (1987) where the seeds were processed after conditioning to 8.0% and 9.0% moisture, respectively. A moisture content greater than 10% may decrease the yield of the fine fractions (Tyler and Panchuk, 1960).
According to Aguilera *et al.* (1982), air classification of bean flour is most efficient when the protein level of the fines fractions is double that of the initial flour. The results with different flours show that protein levels range between 47.0% and 52.1% with good protein recovery values (Tables 2 and 3). The level of protein in protein concentrates of legumes is related to the protein level of the variety or cultivar (Reichert 1982). The relatively low protein level of *Processor* (20.8 g kg^{-1} DM), therefore may account for the protein levels in the fines fractions, which were somewhat lower compared to those reported by previous workers.
Phaseolus vulgaris beans are widely reported to contain lectins and protease inhibitors, which are considered to be the most important bean constituents that exert negative effects when fed to animals (Greer, 1983; Pusztai, 1987). The lectins from *Phaseolus vulgaris* have been studied in great detail. After oral intake these lectins can reach the small intestinal lumen. Their binding to the mucosal surface evokes for instance a degeneration of the intestinal epithelium resulting in villus atrophy and leading to disturbance of normal digestion and absorption and to endogenous nitrogen loss (Kik *et al.*, 1989). Based on the association of lectins and trypsin inhibitors with protein bodies it was expected that these ANF would accumulate in the fines during air classification. For total lectin contents, measured by haemagglutination, an accumulation with the fines during air classification was observed, with values up to three-fold that in the initial flour (Table 4). A comparison of total lectin content determined by two different assays show that, based on ELISA, the lectin activity differs between the two successive fines fractions (Table 5). Based on the haemagglutination assay, however, these fractions show no differences for total lectin content for the two established cut points. This difference may be attributed to the fact that isolectins, containing various proportions of *E*-type and *L*-type subunits (Kik *et al.*, 1989) are not all detected by the haemagglutination assay (Hamer *et al.*, 1989). The lectin tetrameric protein has to contain at least two *E*-type subunits to be detected with the latter assay. In addition, it seems likely, that the apparent discrepancy

lies in the low sensitivity of the HA assay dictated by its reading precision, which is ± one serial dilution.
A correlation of results of the recent ELISA with *in vivo* data has not yet been reported.
Functional lectins have been measured using an assay based on their ability to bind to brush border membrane carbohydrates, with bound lectins being identified using antibodies directed against these lectins (FLIA; Hamer *et al.*, 1989). Based on this immunoassay, functional lectins are concentrated in fines fractions at both cut points after a single pass through the classifier. Levels of functional lectins are lower in FII-fractions following the same distribution pattern observed for total lectins determined by ELISA.
In the literature, many different levels of TIA are reported due to the use of different analytical procedures. In the present study we used a recently developed assay described by Van Oort *et al.*, (1989). Batches of *Phaseolus* beans contained TIA levels of 10.14 (commercial batch), and of 6.46 and 4.46 mg g^{-1}, respectively (Table 4). In pea flour obtained from smooth and wrinkled-type peas, TIA levels of 1.07 and of 2.53 and 2,89 mg g^{-1}, respectively, were obtained (Van der Poel *et al.*, 1989). These values are in line with an earlier ranking of grain legumes in that *Phaseolus* beans may have TIA levels which are reported to be higher than those in faba beans, field peas, lentils, pigeonpeas or chickpeas (Leterme *et al.*, 1989). A pronounced accumulation of TIA in the fines fractions was evident, although the distribution among the first and second fines fraction was affected by the type of milling and by the classifier speed.
The addition of an air-classified fines fraction to the culture medium evoked various morphological changes in cultured explants of small intestinal tissue: a decreased villus/crypt ratio, formation of microvillus vesicles and shortened and irregular positioned microvilli. These effects have been attributed to the lectins present in this bean fraction since intact lectins are reported to bind to the carbohydrate chains of glycoconjugates present in mucus and in the glycocalyx (Pusztai *et al.*, 1981; Kik *et al.*, 1989). The binding may result in a damaging effect on the intestinal epithelium (Kik *et al.*, 1989).
The present studies show that air classification of beans resulted in an increase in protein level of the fines fractions of up to 52.6% compared to the initial bean flour. The results confirm the findings of previous research that air classification is suitable to obtain bean protein concentrates. However, it is not successful in effecting a clear separation of trypsin inhibitory activities and lectins from bean protein. It is notable that both total and functional lectins as well as TIA are accumulated in the fines fractions, resulting in pathological changes in explants of pig small intestinal mucosa exposed to these fractions. In the coarse fraction a substantial reduction in lectin content was obtained.

For feeding practices, utilization of e.g. the fines fractions depends on the relative contribution of lectins and trypsin inhibitors to the negative effects observed with animals fed unheated beans. Since the direct toxic effect of lectins override the importance of any additional nutritional deficiencies of *Phaseolus* beans (Sgarbieri *et al.*, 1982) the bean concentrates cannot be included in diets for monogastric animals without elimination of at least the lectins. Thermal processing of these fractions or heat treatment of beans prior to milling and air classification are ways to establish such an elimination. Dry heat, for example, will reduce lectin activity and TIA (Van der Poel, 1990) but may also reduce the moisture level of beans, which is important in the efficiency of air classification (Reichert, 1982). For fundamental nutritional studies, air classification may provide bean components with high levels of ANF when necessary.

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CHAPTER III

A Small-scale Pressurized Toaster for Upgrading Grain Legumes and Oilseeds

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Abstract

Various heating techniques and processing systems for the thermal upgrading of grain legumes and oilseeds are discussed with relevance to practical and research application.
A modified steam processing equipment was developed for research into product quality with relevance to whole seeds. To relate processing time (t) with different temperatures (or steam pressure), an equipment was designed to enable high temperature/short time (HTST) processing. The installation was equiped with a belt conveyor to define t precisely. This offers the possibility of the investigation of those quantitative effects, which result from changes in processing temperature and time. In a preliminary experiment, whole soya beans were steam processed for different temperature/time combinations. The advantage of pressurized steaming was evident in that the processing time can be considerably shortened as monitored by the analysis of urease activity. In addition, a small hold-up volume has advantages for production as well as maintenance. Some aspects of process optimization in relation to nutritional quality are discussed.

Introduction

The processing of feed legumes and other feed ingredients involves a wide area of research with nutritional and technological aspects. For example, one may adduce the improvement or deterioration of the nutritional value of legume protein after thermal processing. A considerable amount of fundamental research has been focussed on chemical and biochemical characterization of legume protein constituents. This work has included studies on the isolation, identification, characterization and analytical methodology of individual toxic constituents and storage proteins (Sgarbieri and Whitaker, 1982). The location of various bean constituents within the seed have been described by Pusztai *et al.* (1983) and heat sensitivity of some proteins by Wright and Boulter (1980) and Van der Poel (1990).
In general, it has been recognized that the nutritive value of most plant proteins is increased after heating (Liener, 1976). Heat is likely to cause changes in the physical and nutritional characteristics of bean protein through thermal and/or mechanical energies (Mauron, 1972; De Wet, 1982; Bender, 1984). Heating conditions, however, may also cause amino acids to decompose or to react chemically with other compounds (Roach *et al.*, 1967). Thermal processing, therefore, needs to be aimed at making the essential nutrients available with as little damage by heat as far as possible.

In the literature, many studies report on the heat processing of soya beans or soya products with respect to changes of the nutritional value as measured by chemical assays (Kouzeh Kanani, 1985; Rackis *et al.*, 1986). The processing of beans (*Phaseolus vulgaris*) has largely been studied with the aim to improve cooking procedures. The evaluation of the processing equipment and/or the definition of suitable processing conditions for beans to be used in diets for livestock has, however, only rarely been studied.
In the present work processing equipment is discussed with respect to design and practicability for application in the animal feed industry and for research purposes. A pressurized equipment design is evaluated as an alternative to the conventional steam processing methods. In addition, some aspects of process optimization have been discussed.

Thermal processing

Much of the research work on feed legumes and oilseeds and their residues after the removal of oil has been aimed at the effects of thermal processing on the protein quality. This quality is usually increased by heat treatment due to the inactivation of heat-sensitive toxic or antinutritional factors and, in some legume seeds, due to denaturation of the legume protein (Walker and Kochhar, 1982). Overheating on the other hand may cause some damage to protein and prolonged heating may decrease *in vitro* protein digestibility (Sheard *et al.*, 1986; Deshpande *et al.*, 1983). With respect to the influence of processing variables or the optimization of certain processes, it is notable that the results of thermal processing of feedstuffs as measured by chemical assays may have only limited predictive value for growth data or *in vivo* digestibility.
Because the legume seed proteins are located mainly in the cotyledons (Pusztai *et al.*, 1983), processing methods must comprise an "inner body" treatment to establish the desired quality changes.
In practice, various heating techniques and processing systems are applied to a large variety of raw materials. These techniques can be arranged as shown in Table 1. In all techniques, thermal and/or mechanical energy is applied in different combinations to establish the desired specifications of the final product. Most processes given in Table 1 are currently applied to individual animal feed ingredients and/or to whole diets. In general, when applied to single feedstuffs, they aim at thermoinactivation of enzymes, enzyme inhibitors, toxic factors and the simultaneous reduction in total microbial infestation. The application of steam explosion, pressure cooking and extrusion further aim at product alterations in such a way, that both structure and texture of the processed product are influenced (Van Zuilichem and Stolp, 1989).

Table 1. Thermal processing techniques and operating conditions.

Process	Design[1]	Decisive factors				Heating Source	Type[2]	Tpt[3]
		Temp. (°C)	Time (sec)	Steam	Pressure			
Extrusion cooking	HT-ST	80-200	30-150	+/-	high	steam/ friction	C	M/L
Expander	HT-ST	80-140	100-200	+	medium	steam/ friction	C	M/H
Infrared irradiation	HT-ST	80-130	40-60	-	-	natural gas	C	M
Steam processing								
- steam plosion	HT-ST	140-210	20-45	+	high	steam	B	L
- autoclaving	HT-MT	110-130	600-1000	+	medium	steam	B	L
- toasting (conventional)	LT-LT	90-105	1800-2700	+	low	steam	C	M/H
- toasting (high pressure)	HT-ST	100-140	60-300	+	medium	steam	C	M/H

[1] H(L)T; high (low) temperature - S(M)(L)T: short (medium)(long) time
[2] C = continuous-type; B = batch-type
[3] Throughput: (H)igh, (M)edium, (L)ow

Different conditions are obtained by varying decisive (product and) processing variables such as temperature, time of exposure, moisture content and, eventually, pressure and shear (Seerley *et al.*, 1974; Rackis *et al.*, 1986; Van Zuilichem *et al.*, 1980a). The extent to which the characteristics of the final product are altered is a function of these variables whereas some of these variables are interrelated, depending on the equipment design. For example, the temperature which is stationary at a certain fixed point in an extruder can be a function of time.

With respect to time-temperature conditions two processes, long-term and short-term can be identified. In general, low temperature, long time (LTLT) processes operate with temperatures at about 105°C and processing times in the range of 10 to 45 min. High temperature, short time (HTST) designed processes involve temperatures up to 210°C with exposure times of 20 to 300 seconds. Therefore, the restrictions for the use of certain steam processing equipment can be deduced from the general relationship between the residence time and pressure (and related temperature) exerted in the equipment. HTST processing is considered preferable in terms of nutrient retention, since antinutritional factors and contaminating micro-organisms are destroyed more effectively (Björck and Asp, 1983).

Extrusion cooking and the expander process are among the novel processes. In principle, the extruder/expander are types of "screw machinery" in which the product is crushed, mixed, heated, pressurized and expanded at the outlet. This equipment, therefore, cannot be considered as a single operation because it performs a large number of functions. These include mixing, thermal treatment, shaping, sterilization and partial drying. In this kind of equipment, thermal energy is supplied and, simultaneously, mechanical shear is dissipated as heat. In addition to thermal energy the use of shear implies a process variable which itself may change the properties of the product. The latter change varies also with the different kind of extruders/expanders being applied. In this regard, less information is available from studies on e.g. twin-screw extruders and advanced screw configurations compared to single-screw extruders (Van Zuilichem *et al.*, 1980a; Björck and Asp, 1983). For the influence of extrusion cooking on the nutritional value see reviews of Harper (1979), Björck and Asp (1983) and Asp and Björck (1989).
Infrared (IR) radiation involves the exposure of particle solids to IR radiation for a short period of time. This results in a rapid rise of temperature and an increase in water vapour pressure in the product. The equipment used is referred to as a "micronizer" (Livingstone, 1977). However, in order to use the residual heat of the heated product leaving the conveyor belt of the IR plant the equipment has been redesigned as described previously (Van Zuilichem *et al.*, 1980b). In the latter procedure the product is held for a predetermined period in an insulated container after short-term radiation. The residual heat will equilize both by conduction and by diffusion of heat and further act to achieve the processing objectives (Kouzeh Kanani *et al.*, 1981). IR radiation has been the subject of several studies on various agricultural products (Kouzeh Kanani, 1985; Van Zuilichem *et al.*, 1985).
A large part of heat treatments is concerned with steam driven equipment of different kind, either batch or continuous operated (Table 1).

Steam processing equipment

The use of steam is convenient because of the attractive heat transfer coefficient and the amount of condensation heat. It is assumed that direct steaming, also at higher temperatures, includes rather mild conditions due to the effect of steam condensation on the surface of the outer layer of seeds. This provides some kind of protection against overheating.
Steam heating at about 100°C, a process generally termed "toasting", is commonly applied in the animal feed industry as an economical treatment for e.g. legumes and some oilseeds. This application involves the use of

conventional vertical (Cascade-type) toasters. This type of toaster is normally used in the oil extraction industry (Kaufmann *et al.*, 1965). Flakes of oilseeds such as soyabeans are extracted with hexane to remove the oil. For feed use, the flakes are desolventised in a desolventiser-toaster to remove the hexane efficiently as well as to inactivate ANF.

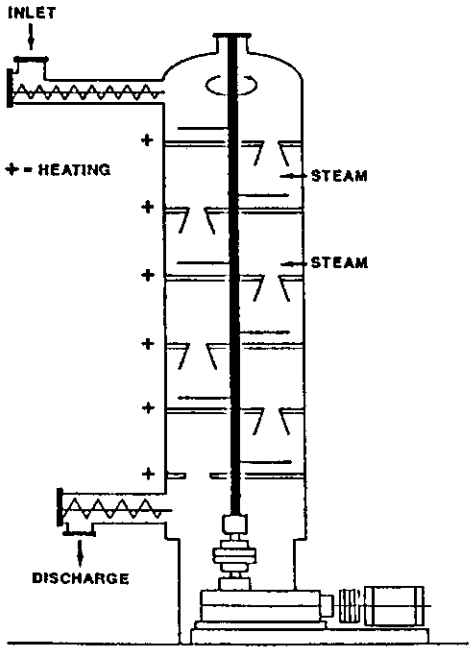

Figure 1. Cascade-type toaster (For explanation, see text)

The conventional toaster (Figure 1) represents a piece of equipment, vertically built, with several decks and intermediate floors. Heat is supplied by direct steam addition in the upper decks and, furthermore, both walls and floors can be heated. The product enters the toaster and it passes several decks in a continuous flow by means of stirring devices. The cascade-type toaster has some limitations with respect to the level of pressure and, therefore, the temperature involved is relatively low (about 100°C). With this temperature, low reaction rate constants are found for the inactivation of antinutritional factors (Van der Poel *et al.*, 1990). Consequently, longer processing times are needed. Prolonged heating, however, may decrease the amount or availability of essential amino acids such as lysine. Also, it is assumed that the residence time distribution of the particles in this type of equipment is not very attractive.

The processing objectives for upgrading legume seeds or oilseeds in terms of e.g. ANF inactivation can only be established in much shorter times when heating levels are higher. This can be performed in pressurized equipment. Under the assumption that the inactivation of ANF may be described by a first order reaction (Rackis *et al.*, 1986; Van der Poel, 1990), the use of an Arrhenius model is justified for the temperature dependency of the reaction rate constant k:

$$\text{Ln}k = \text{Ln}k_o - (E_a/R)(1/T) \qquad \text{Eqn (1)}$$

where k_o is a constant, E_a is activation energy, R the gas constant and T the absolute temperature. From Equation 1 it is obvious that the reaction rate constant increases at higher temperatures.

To study the effects of thermal treatment on nutrients at high temperatures, autoclaving has generally been used. Despite long investigations, the equipment for autoclaving comprises a rather unpractical (batch-type) design with respect to handling. Moreover, processing times cannot be well defined since time is needed for preheating the material to establish a certain pressure level. Furthermore, considerable time is needed for cooling before the product can be removed. In addition, steam consumption for this batch-type technique may be unfavourable.

Due to inadequate assessment of certain processing variables and to some practical disadvantages, described above, conventional steam treatments as well as autoclaving are rather inconvenient to use for systematic studies. For this objective, residence times have to be defined and controlled carefully. Therefore, a laboratory-scale pressurized toaster was developed to provide a continuous and HTST-designed process and to provide controlled conditions.

Laboratory-scale pressurized toaster

A laboratory-scale toaster (Figure 2) for batch-type or continuous steaming was developed at the Agricultural University. The equipment consisted of a horizontal cylindrical vessel with a built-in steel-woven belt conveyor. The conveyer can operate at different speeds, allowing different residence times and throughputs. The vessel was provided with a loading and a discharge sluice to operate at internal (absolute) pressures ranging from 100 to 400 kPa. The loading or feed sluice (fs) has a variable speed drive to influence the layer thickness of the product on the conveyor. The discharge sluice (ds) has a fixed speed. The process heat is supplied by direct steam injection and temperatures can be determined at three points using thermocouples.

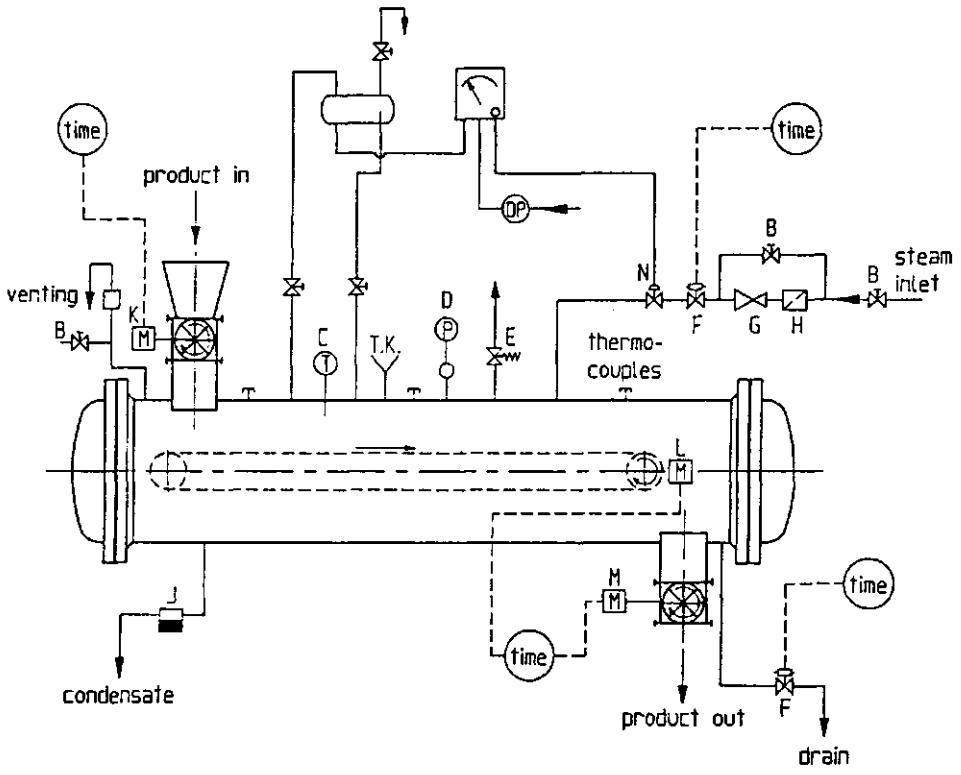

Figure 2. Laboratory-scale pressurized toaster (Agricultural University; for explanation, see text)

The process variables such as feed rate, steam pressure and conveyor speed can be controlled automatically. Steam condensate produced during processing is drained. Applying a belt conveyor, the residence time can be adequately determined.

Experiments

Some experiments were carried out in order to record the dependency of processing variables and control settings for steam processing. The relationship between steam pressure and temperature was established and residence times were recorded at various control settings for the feed sluice and conveyor, respectively. In addition, the steam heating effectiveness was investigated.

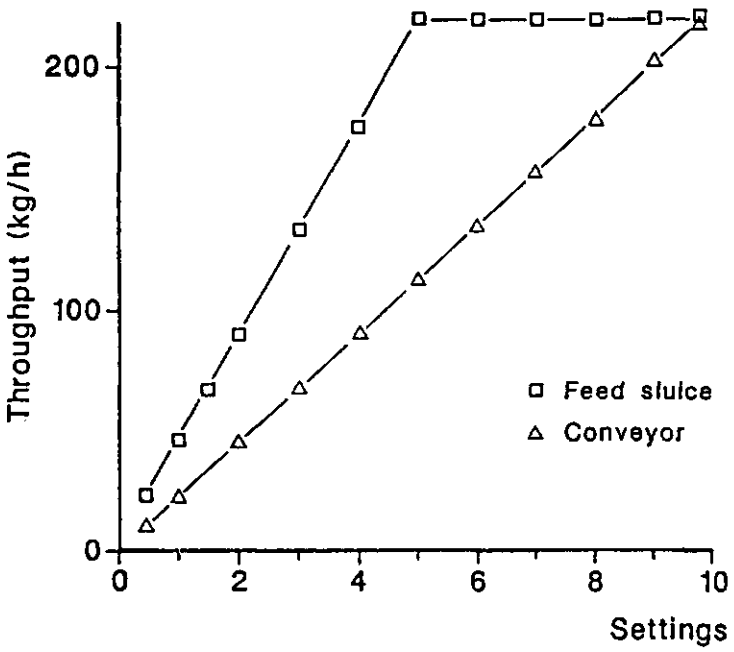

Figure 3. Throughput (Φ; kg/h) of the feed metering sluice (fs) and conveyor (c) as a function of the control settings (S)

Temperature/steam pressure - Residence time. Temperatures were measured for different steam pressures at three points using thermocouples. Although some pressure is lost in the system, the pressure levels recorded showed a good correlation with the mean values of the recorded temperatures. Both residence time and system throughput vary with the control settings (S) of the equipment. Initially, the throughputs (Φ; kg/h) were determined for the feed sluice (fs) and the conveyor (c), respectively (Figure 3). In addition, the residence time (t; sec.) was measured based on equal throughputs of the feed sluice and conveyor (Figure 4). These measurements may be summarized by the following equations:

Φ_{fs}	=	$2.54 + 45.0\ S_{fs}$	(S_{fs}, 0-5 ;	R^2=0.98)	Eqn (2)
Φ_c	=	$2.72 + 23.0\ S_c$	(S_c , 0-10;	R^2=1.00)	Eqn (3)
t	=	$t_{fs} + t_c + t_{ds}$			Eqn (4)
t_{fs}	=	$(1/3.61\ S_{fs}).10^3$		(R^2=0.96)	Eqn (5)
t_c	=	$(1/1.41\ S_c).10^3$		(R^2=0.96)	Eqn (6)
t_{ds}	=	5			

With respect to the processing time at high temperatures, it is notable that the time, needed to elevate the mean temperature of the product is meaningfull and can be considerable with respect to very short processing times.

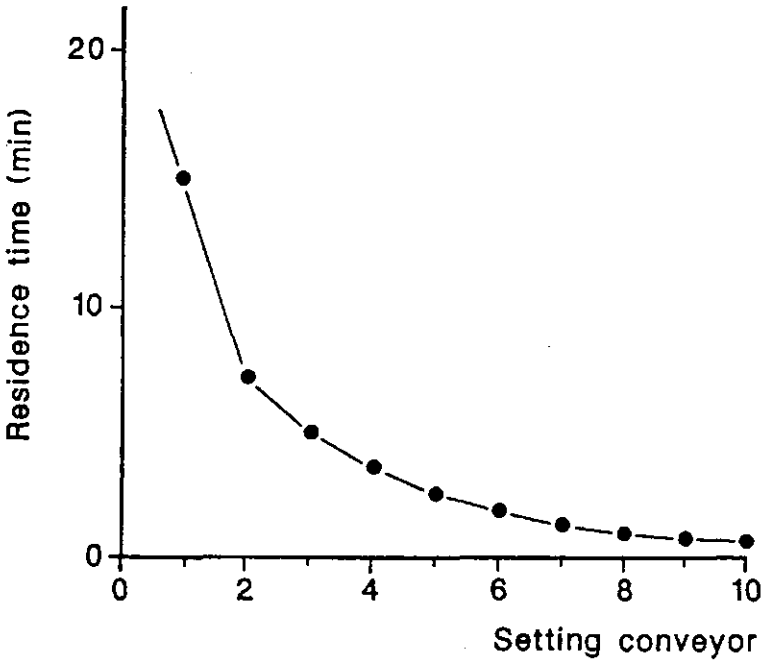

Figure 4. Product residence times (t, sec) for a 'steady state' situation ($\Phi_c = \Phi_{fs}$)

Steam heating effectiveness. The effectiveness of the equipment with respect to steaming at ~100°C and to pressurized steaming was investigated by the heating of whole soya beans (*Glycine max*). The soya beans, grown in Argentina, were purchased from a commercial supplier. The beans were cleaned by air aspiration and subjected to steam heating at temperatures (T) of 102, 120 and 137°C. Different processing times (t) were examined being defined as the summation of the residence time on the conveyor belt and in the discharge sluice. Samples of soya beans were steam processed in a randomized order. Processed beans were dried and were analysed for urease activity (ΔpH-rise; AOCS, 1979) which is commonly used as an indicator for the degree of heat processing and, somewhat arbitrarily, for trypsin inhibitor activity (TIA).
The values for residual urease activity in the soyabeans after steaming are presented in Figure 5. From these data it can be seen that steaming at 102°C inactivates the urease activity to the low level of 0.02 units pH-rise after 40

min. For similar residual levels, steaming at 120 and 137°C requires only 10 and 1.5 min., respectively.

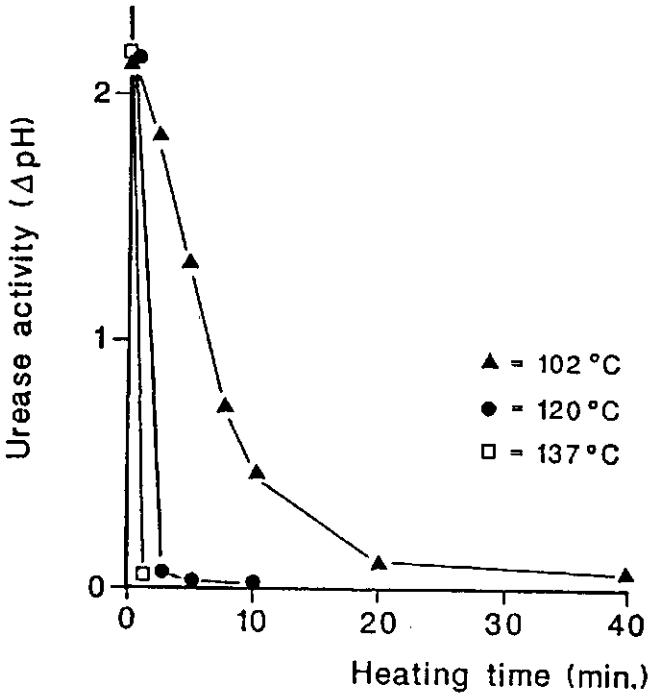

Figure 5. Inactivation of urease activity during steam processing at different temperatures

Scaling-up

For industrial application, up-scaling is necessary to meet the requirements for higher throughputs. Since the design of the pressurized toaster includes a small volume, the dimensions can easily be optimized at each pressure level for mechanical engineering purposes using the following relationships:

$$V = \pi.d^2/4 \,.\, l \qquad \text{Eqn (7)}$$

$$l = v_c \,.\, t \qquad \text{Eqn (8)}$$

$$V = \pi.d^2/4 \,.\, v_c \,.t \qquad \text{Eqn (9)}$$

where V is equipment volume, d is interior diameter, v_c is conveyor speed, l = length and t is processing time. Equation 9 shows the toaster design as a function of processing time and conveyor speed, whereas the optimum processing times are about $60 < t < 300$ sec.

Process optimization

The quantitative approach to the assessment of product quality changes during processing has gained surplus value by the increase in knowledge from kinetic studies based on advanced, sensitive analytical assays. In general, this analytical approach allows a wide scope of research. It may provide alternative processes and process conditions to minimize undesired changes such as decreased availability of amino acids and to optimize nutrient availability and utilization (Saguy and Karel, 1980).

The various chemical assays used to predict nutritional value, however, cannot always be considered as unique substitutes for biological appraisal of protein value with relevance to the utilization of the protein in animal nutrition (Donatucci *et al.*, 1987). According to Burns (1987), for example, measurements of trypsin inhibitor activity alone cannot be used to assess protein quality, as other factors are also involved such as amino acid availability and protein digestibility. Furthermore, chemical methods cannot distinguish between a low nutritional value obtained in biological assays as a result of either poor protein quality or slight toxicity of processed beans (Bender, 1984). The exploration of the effects of thermal treatments based only on chemical assays and the subsequent scientific explanation of these results (Figure 6), therefore, has to be followed by a biological assay prior to an optimization of processing conditions.

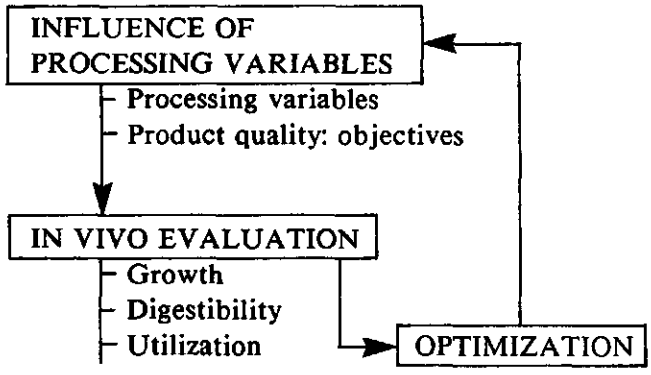

Figure 6. Diagram for optimization of thermal processing conditions for animal nutrition purposes

This is likely to be of primary concern in evaluating protein nutritional value of heat processed grain legumes, oilseeds and oilseed byproducts in which residual levels of ANF or toxic constituents are still present and may exert slight negative effects when fed to animals.

Conclusions

A simple and effective toaster has been designed providing a HTST-treatment for continuous and batch-type pressurized steam processing and has been tested. Based on the evaluation of a preliminary experiment it appeared that the proposed procedure for HTST steaming may have several important advantages. Contrary to the conventional kaskade-type toaster, the pressurized toaster has a small hold-up volume which is convenient with respect to production and maintenance. The residence time distribution is attractive and this type of toaster permits the use of short processing times. Consequently, high throughputs are obtained.
Finally, it is concluded that studies directed towards the effect of thermal processing on product quality at least need a validation in a biological assay. Both kinetic data and data from animal performance can subsequently be used for a reliable process optimization. Especially the introduction of new feed processing methods (twin-screw extruders, expanders, pressurized steaming) calls for consideration in this respect.

Acknowledgements

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CHAPTER IV

Thermal Inactivation of Lectins and Trypsin Inhibitor Activity during Steam Processing of Dry Beans (*Phaseolus vulgaris* L.) and effects on Protein Quality.

A.F.B. van der Poel, J. Blonk, D.J. van Zuilichem and M.G. van Oort

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Abstract

The effect of steam treatment on the protein quality and antinutritional factors in beans (*Phaseolus vulgaris* L.) have been evaluated. The thermal inactivation of total and functional lectins and trypsin inhibitor activity as well as total and available lysine during steam treatment at 102, 119 and 136°C can be described by first-order reaction kinetics. Inactivation of TIA occurred in two stages with different reaction rates, with the initial stage having a higher rate of inactivation. The effect of steaming temperature on the rate constants can be predicted by an Arrhenius type relation.
A part of the total lysine was lost on heating but the amount of available lysine was reduced to a greater extent. The results of the present investigation indicate that steam treatment at 119°C for 5 or 10 minutes seems to be a good compromise in terms of ANF inactivation and protein damage as measured by total and available lysine. Further research is required to evaluate the effects of steaming at 136°C for <1.5 minutes.

Introduction

Seeds of *Phaseolus vulgaris*, either raw or inadequately processed, are toxic when ingested by humans or monogastric animals (Noah *et al.*, 1980; Pusztai, 1980). These beans contain a wide range of nutrients and antinutritive and/or toxic components of different composition. Lectins appear to be the most deleterious antinutritional factors (ANF) (Pusztai *et al.*, 1979; Liener, 1980). The trypsin inhibitory activity of these beans is also relatively high. It has been well established that the nutritive value of *Phaseolus* bean protein is enhanced by thermal processing, especially by moist heat treatments (Gallardo *et al.*, 1974). This may be due to denaturation of proteinaceous ANF because these factors require their structural integrity in order to exert their negative *in vivo* effects (Burns, 1987). Moreover, the increased nutritional value may be the result of an increased accessibility of bean proteins to enzymatic attack (Romero and Ryan, 1978). The heat process must guarantee sufficient inactivation of ANF while significant degradation of essential amino acids must be avoided.
The effectiviness of thermal processing of a given product depends on a combination of temperature, time and the moisture level and particle size. Therefore, a HTST-designed process (high temperature-short time) may give different results compared to a long-term process, operating at lower temperatures (LTLT).

Several studies have investigated the thermal inactivation of total lectins and protease inhibitor activities as a function of time and temperature (Van der Poel, 1990). Apart from cooking procedures (fully hydrated beans), the kinetics of thermal inactivation in whole beans of lectins and protease inhibitors in relation to essential nutrients (amino acids) are rarely studied in a systematic way. Moreover, assays have now become available for the determination of functional lectins (Hamer *et al.*, 1989).
In this investigation, the laboratory-scale steam processing of beans was evaluated. Analytical aspects of bean proteins and antinutritional factors, including functional lectins, were investigated. In addition, the influence of different operating conditions of steam processing on these constituents were assessed.

Materials and Methods

Dry common beans (*Phaseolus vulgaris* L., cull grade), grown in the USA, were purchased from a commercial supplier. The batch was composed of about 12 types of beans with different colour, size and shape. The batch was characterized for some physical properties (split beans, water absorption index), for chemical composition (proximate analysis), foreign matter and the level of aflatoxin B1 (Table 1). Beans were not crushed before thermal processing.

Table 1. Physico-chemical characteristics of the *Phaseolus vulgaris* beans.

Chemical		
Dry matter	87.00	(%)
Ash	4.45	(% DM)
Crude protein (N x 6.25)	27.42	(% DM)
Nitrogen-free extract	62.18	(% DM)
Crude fibre	4.04	(% DM)
Ether extract	1.90	(% DM)
Physical		
Number of bean types	12	
Foreign matter	13	(g/kg^{-1})
Split beans	63.2	(%)
Water abs. capacity	26-69	(ml/g^{-1})[1]
Aflatoxin B1 level	9.4	(mcg/kg^{-1})

[1]Range: soaking time 5 min. - 120 min.

Processing

A laboratory-scale pressurized toaster (Figure 1) was developed at the Agricultural University for batch-type or continuous steaming. Throughout the experiments, the toaster was used for batch-type direct heating of the beans. The machinery consisted of a horizontal cylindrical vessel with a built-in steel-woven belt conveyor. The conveyor can operate at different speeds, allowing different residence times and throughputs. The vessel is provided with a loading and a discharge sluice. The loading sluice has a variable speed drive to influence the layer thickness of the product on the conveyor. The discharge sluice has a fixed speed. The process heat is supplied by direct steam injection and temperatures can be determined at three points using thermocouples. Process variables (feed rate, internal pressure and conveyor speed) were controlled automatically. Steam condensate produced during processing was drained. The toaster has been designed to operate at internal (absolute) pressures ranging from 100 to 400 kPa.

After steaming to the desired temperature, batches of 3 kg of beans were metered in the inlet sluice. This sluice was operated at a feed rate providing a single layer of beans on the belt conveyor. The batch was held for different residence times under specific heating conditions with maximum temperature tolerances of 0.5°C being attained.

After steam processing all samples were immediately air-dried (35°C for 24 hours) prior to milling (stepwise: 6 and 1 mm, resp.), storage (4°C) and analysis.

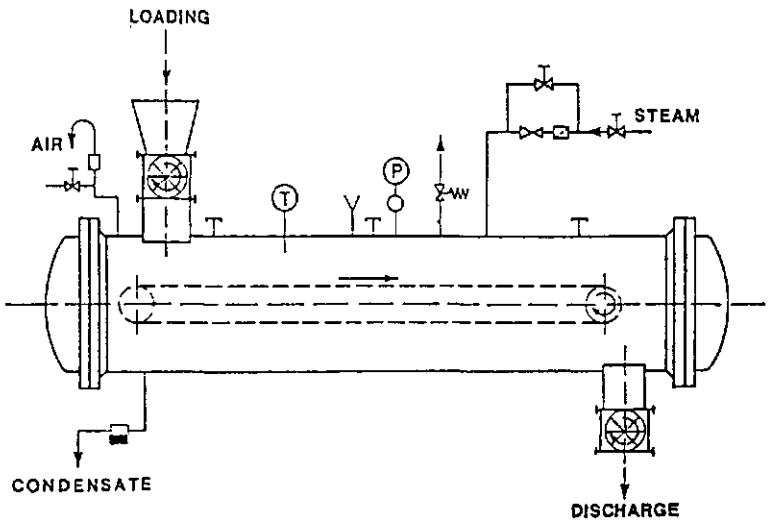

Figure 1. The laboratory-scale toaster. Operating conditions: P_{max} = 400 kPa; T_{max} = 143°C; V = 105 l.; 'T' = thermocouple; P = pressure gauge.

Experimental design

Steam heating of common beans was performed at atmospheric conditions and at two higher pressures (300 and 400 kPa, absolute) for different processing times (Table 2). The residence time (t) was defined as the summation of the residence time of the beans on the conveyor and the residence time in the discharge sluice (5 sec., fixed). Samples of beans were steam processed in a randomized order and analysed for moisture and nitrogen content. In addition, levels of lectins and trypsin inhibitor activities and protein quality parameters were analysed.

Four samples were used to determine the effect of storage of processed beans on the possible reversibility of lectin protein inactivation. For this experiment beans were packed in plastic bottles and stored at room temperature for seven weeks.

Table 2. Processing conditions for steam treatments

Steam pressure (kPa)	Temperature (°C)	Processing time (min.) 1.5	2.5	5	10	20	40	60	80	120
100	102			*	*	*	*		*	*
300	119		*	*	*	*	*	*		
400	136	*	*	*	*	*				

Analytical methods

Chemical and physical analyses were carried out in duplicate on air-dried samples (milled to pass a 1 mm screen) unless otherwise indicated.

Dry matter, crude fibre, crude fat (DEE) and ash were analysed by standard methods (Weende analysis). Nitrogen was determined by the Kjeldahl method (ISO, 1979) and a nitrogen to protein conversion factor of 6.25 was used.

The level of aflatoxin B1 was determined in the fresh sample. The extraction and purification procedures were according to AOAC (1984); separation according to Traag *et al.* (1987). Foreign matter and split bean percentages were determined by manual separation of 300 g samples. Water absorbtion capacity (WAC) of beans was determined according to the method reported by Esaka *et al.* (1987).

The effect of heating on protein quality was estimated by determining the Protein Dispersibility Index (PDI; AOCS, 1964) and the available lysine value (ALV; Hall *et al.*, 1973). For ALV determinations, samples were re-milled to

pass a 0.08 mm screen. In addition, the content of total lysine (TL) was determined using ion-exchange chromatography. The analysis was conducted on a Biotronic LC 2000 amino acid analyzer.

Total lectin content was determined by measuring the haemagglutination activity (HA; units g^{-1}) with rabbit red blood cells (Valdebouze *et al.*, 1980) and by an ELISA-method using antibodies raised in rabbits against the total lectin fraction of *Phaseolus* beans (Hamer *et al.*, 1989). Analysis for the content of functional lectins was carried out by an immunoassay for functional lectin (FLIA) as described by Hamer *et al.* (1989). The FLIA was performed with a coating of porcine small intestinal brush border membrane (FLIA-BBM) or a coating was used of fetuin albumin conjugate (FLIA-Fetuin). These different analyses were performed due to the difficulty of quantifying the contents of (iso)lectins in legumes seeds and the possible relationship with future *in vivo* data.

All ELISA and FLIA values were determined in quadruplicate and lectin contents are expressed relative to purified *Phaseolus* lectins (mg g^{-1} product). Trypsin inhibitory activity (TIA; mg g^{-1} product) was measured in re-milled samples (0.08 mm) according to a modification of the method of Kakade *et al.*, (1974) as described by Van Oort *et al.* (1989).

Curve fitting

Linear regression by the least-squares method was used to fit the inactivation data of total and functional lectins, TIA and other parameters to the kinetic model given by equation (1). The reaction rate constant k was calculated for the parameters from the slope of the curve that represents the relationship between the logarithm of relative contents (C/C_0) and thermal processing time (t). The effect of temperature on the rate constants was calculated from the Arrhenius equation (eqn (2)). Values for PDI were observed to increase after some heating and these values were excluded for the calculation of the rate constants.

$$\ln (C/C_0) = -k.t \qquad \text{Eqn (1)}$$

$$\ln k = \ln k_0 - (E_a/R)(1/T) \qquad \text{Eqn (2)}$$

Results

Steam heating

The analytical data pertaining to the steam processed beans at different pressures and processing times are presented in Tables 3 (100 kPa, 102°C), 4 (300 kPa, 119°C) and 5 (400 kPa, 136°C).

Table 3. Analytical data (% DM) of beans after atmospheric steaming (100 kPa; 102°C)

	Untreated (C_o)	Processing time (min.) (C)					
		5	10	20	40	80	120
Air dry matter (%)	94.1	87.8	88.7	87.4	84.5	79.9	80.4
Dry matter (%)	87.0	80.1	80.9	79.8	77.1	72.8	73.6
Crude protein (%)	27.5	27.4	27.6	27.3	27.3	27.4	27.3
TL ($g/16g^{-1}$ N)	6.32	6.50	6.44	6.55	6.40	6.40	6.29
ALV ($g/16g^{-1}$ N)	5.36	5.35	6.19	5.37	5.28	5.04	4.78
PDI (%)	34.2	22.0	20.8	20.1	18.6	17.4	16.9
Lectins							
HA (%)	100	100	156	31	31	0.02	<0.02
ELISA (%)	100	43.4	35.7	18.3	5.4	1.1	0.1
FLIA-BBM (%)	100	41.8	34.0	20.0	2.4	0.2	0.2
FLIA-Fetuin (%)	100	78.8	69.6	34.1	10.5	2.9	<0.4
TIA (%)	100	34.2	21.5	13.7	9.4	4.1	1.3

Table 4. Analytical data of beans after steaming at 200 kPa (119°C)

	Untreated (C_o)	Processing time (min.) (C)					
		2.5	5	10	20	40	60
Air dry matter (%)	94.1	86.4	85.0	83.4	82.5	80.1	79.4
Dry matter (%)	87.0	79.0	77.6	76.1	75.2	73.0	72.3
Crude protein (%)	27.5	27.5	27.6	27.4	27.5	27.3	27.3
TL ($g/16g^{-1}$ N)	6.32	6.48	6.37	6.40	6.34	6.13	6.06
ALV ($g/16g^{-1}$ N)	5.36	5.56	5.09	5.22	4.91	4.40	4.55
PDI (%)	34.2	19.6	17.3	15.1	15.1	15.7	16.4
Lectins							
HA (%)	100	25	15	1.5	0.8	*	0.8
ELISA (%)	100	5.2	1.4	0.08	0.04	0.09	0.02
FLIA-BBM (%)	100	1.7	<0.4	<0.4	<0.4	<0.4	<0.4
FLIA-Fetuin (%)	100	12.6	3.5	<0.4	<0.4	<0.4	<0.4
TIA (%)	100	11.2	6.3	3.6	1.9	0.7	0.6

* No HA detected

Steaming does not influence the protein content of the bean irrespective of treatments. Steaming, however, rapidly reduced the dispersibility (PDI) of bean proteins from 34.2% (initial level) to 16.9% at 100 kPa; 120 min. and 15.1% for both 300 kPa; 10 min. and 400 kPa; 2.30 min. The latter conditions for steaming showed an increase for PDI with prolonged heating after reaching the minimal level (Tables 4 and 5). The pattern for the change of PDI during heat treatment is biphasic and analogous to TIA, except at lower rate constants (Table 6; Figure 2). Rate constants for loss of ALV and total lysine were relatively low (Table 6).

Table 5. Analytical data of bean after steaming at 400 kPa (136°C)

	Untreated (C_o)	Processing time (min.) (C)				
		1.5	2.5	5	10	20
Air dry matter (%)	94.1	81.7	82.7	84.5	82.0	79.0
Dry matter (%)	87.0	74.9	75.9	77.5	74.8	71.9
Crude protein	27.5	27.5	27.3	27.5	27.4	27.6
TL (g/16g^{-1} N)	6.32	6.47	6.46	6.32	6.14	5.69
AVL (g/16g^{-1} N)	5.36	4.68	4.52	4.25	4.34	3.23
PDI (%)	34.2	15.2	15.1	15.5	16.4	18.2
Lectins						
HA (%)	100	0.8	*	*	0.08	8
ELISA (%)	100	0.14	0.09	0.03	<0.02	<0.02
FLIA.BBM (%)	100	<0.4	<0.4	<0.4	<0.4	<0.4
FLIA-Fetuin (%)	100	<0.4	<0.4	<0.4	<0.4	<0.4
TIA (%)	100	5.7	2.0	1.8	0.5	0.6

*No HA detected

Absolute ANF contents of the untreated beans were as follows: HA, 64; ELISA, 58; FLIA-BBM, 52; FLIA-fetuine 55.4; TIA, 11.0. Lectin contents, amount of functional lectins and TI activity of beans were reduced by steam treatment at various temperatures as shown in Tables 3-5 relative to the untreated material.

Table 6. Reaction rate constants (k_r, sec^{-1}) and activation energies (E; kJ mol^{-1}) for several parameters at 102, 119 and 136°C.

	Temperature (°C)			Activation energies (kJ mol^{-1})
	102	119	136	
Trypsin inhibitor				
*k*1	12.88	52.56	117.34	82.4
*k*2	1.46	2.46	7.67	61.9
Lectins				
HA	3.38	13.87	226.51	156.9
ELISA	3.23	22.07	175.78	149.4
FLIA-Fetuin	3.00	36.39	248.63[1]	160.8
FLIA-BBM	4.56	69.71	248.63[1]	152.7
PDI				
*k*1	5.30	13.34	20.58[1]	51.2
*k*2	0.11	1.01	20.58[1]	195.0
Lysine	0.0108	0.0588	0.369	103.3
ALV	0.078	0.213	1.26	132.1

[1]Only a single value was calculated for *k*1, *k*2 at 136°C.

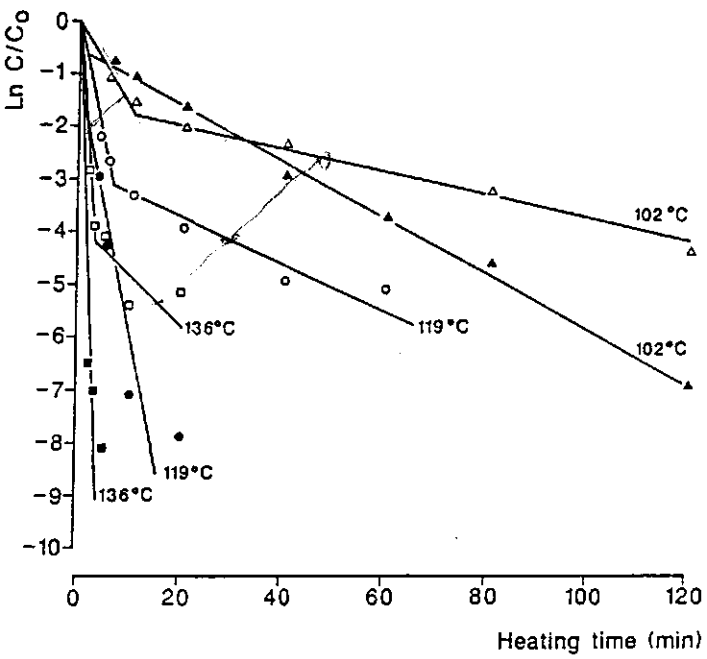

Figure 2. Effect of processing time on the inactivation of lectins (ELISA; ▲, ●, ■) and TIA (△ , ○ , □) after heating at three different temperatures.

At atmospheric conditions the haemagglutination activity (HA) of beans is increased after 10 min. of steaming but is considerably decreased thereafter to a minimal level of 0.1 units after 120 min. Lectin contents measured by both ELISA and FLIA show a different pattern of inactivation in which the activity is constantly decreased by the successive processing times. At 300 and 400 kPa the initial short heating times reduce lectin activity to a large extent. Kinetic parameters for ANF thermal inactivation are presented in Figures 2-4 and in Table 6. All parameters are well described following first-order kinetics.

For atmospheric steaming the inactivation of total lectins (ELISA) follows almost a same pattern as functional lectins measured by FLIA-BBM. It is noteworthy that heat inactivation (100 kPa) of functional lectins measured by FLIA-fetuin follows a similar pattern as lectins assayed by ELISA or FLIA-BBM, but at a much lower rate (Figures 2 and 3). During thermal inactivation, the residual total lectins (ELISA), as a function of time, show a different curve as compared to the residual TIA (Figure 2). Inactivation of lectins could be described with first-order kinetics. The rate of lectin loss increases at higher temperatures, being highly temperature dependent.

The plot of PDI values and of TIA inactivation, however, did not conform to a single reaction following first-order kinetics in that a dual rate behaviour was found at all temperatures. The initial and latter parts of the TIA curves are linear but clearly show a different slope. This can be interpreted by the occurence of two concomitant (first-order) reactions with rate constants *k1*

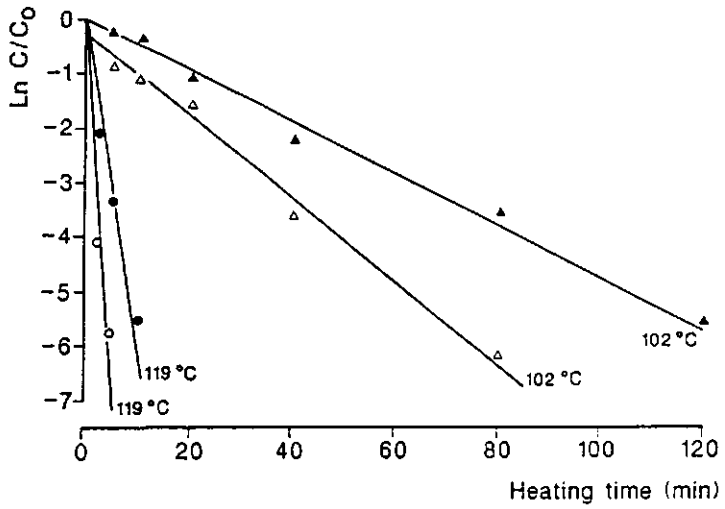

Figure 3. Inactivation of functional lectins (○ , △ , FLIA-BBM; ● , ▲ , FLIA-Fetuin) after heating at 102°C and 119°C.

and *k2* where *k1* >> *k2*.

In order to determine the influence of temperature on the levels of the nutritional parameters investigated, Arrhenius plots (Ln K_r vs T^{-1}; Table 6) and graphs for TIA/PDI (Figure 4) were made. Increasing the temperature increased the rate constants k_1 and k_2 for both TIA and PDI values, k_2 (PDI) having a somewhat different slope. This means that the thermal resistance of TIA becomes less at higher temperatures.

For beans processed at 102°C, a slight reversibility of inactivation of total lectins (ELISA) was observed after storage for 7 weeks (Table 7). The content of lectins, measured according to FLIA-fetuin, however, was clearly decreased. Beans treated at temperatures of 136°C did not show any change in lectin contents after storage.

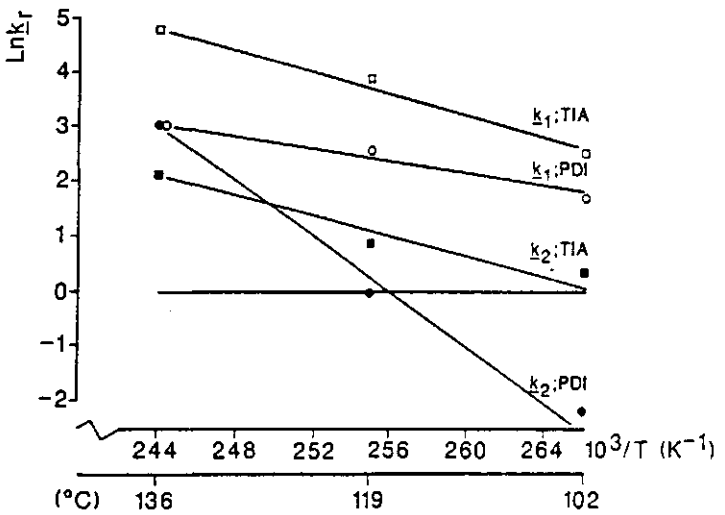

Figure 4. Arrhenius plot of the first and second stage of altering TIA and PDI values (R^2: *k*1, TIA 0.98; *k*2, TIA 0.95; *k*1, PDI 0.97; *k*2, PDI 0.99).

Discussion

For feeding purposes, beans of *Phaseolus vulgaris* have to be processed to eliminate their constituent lectins which are the toxic factors in these beans (Grant *et al.*, 1982). This can be done by thermal treatment which also reduces the activity of other nutritionally undesirable heat sensitive substances. The

Table 7. Reversibility of thermal inactivation of (functional) lectins[1] after steam treatment at 102 and 136°C and subsequent storage (weeks).

	ELISA		FLIA-Fetuin		FLIA-BBM	
	0	7	0	7	0	7
102°C, 20 min.	18.3	22.6	34.1	4.4	20.0	19.4
102°C, 40 min.	5.4	6.5	10.5	<0.4	2.4	4.1
136°C, 2.5 min.	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
136°C, 5 min.	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4

[1] %; relative to untreated beans

treatment must keep protein deterioration to a minimum. Therefore, processes applicable to the feed manufacturing industry necessitate an accurate knowledge of the changes in bean constituents by thermal treatments.
In practice, the presence of a single bean type cannot be guaranteed. This implies the occurence of different seed lines in a single batch, each of which have different levels of nutrients and antinutritional factors. In the present investigation the choice of a commercial batch, containing several types of beans, was made to establish conditions analogous to the practical feed industry.
Analysis of trypsin inhibitor activity and total lectins (HA) in the untreated beans revealed high levels (10.14 mg g^{-1} and HA of 64) compared to other grain legumes (Leterme *et al.*, 1989). It has been established that these proteinaceous ANF in beans are inactivated to a large extent during thermal treatments (Boufassa *et al.*, 1986; Rackis *et al.*, 1986; Roa *et al.*, 1989; Van der Poel, 1990). The inactivation of lectins in the present study was monitored by using four different assays offering specificity and sensitivity for the detection of *Phaseolus* isolectins (tetramers; *L4*, *L3E*, *L2E2*, *LE3*, *E4*; Leavitt *et al.*, 1977). These assays are based on the affinity for different tetramers. The ELISA technique measures all tetramers, due to the cross-reactivity of *E* and *L*-type anti-lectin antibodies. The FLIA-BBM assay is indicative for *L4*, *L3E*, *L2E2* and *LE3* while FLIA-fetuin does not react with the *L4* tetramer. The haemagglutination assay, finally, is indicative for all tetramers with at least two *E*-type protomers.
For all lectin tetramer combinations, thermal inactivation followed a first-order reaction at the temperatures investigated. The effect of processing

temperature on the rate constants can be predicted by an Arrhenius type relationship. It is noted that, at a temperature of 136°C, the reliability of the rate constants is not optimal due to the smaller discrimination of the assays used for monitoring the low residual contents. With the different assays for lectins, the lowest rate constants were observed for activities measured with FLIA-fetuin (at 100 kPa) and with ELISA (at 300, 400 kPa). The latter assay seems to be a most sensitive assay for measuring total lectin content at low levels. However, this assay does not distinguish between functional and non-functional lectins. Boufassa *et al.*, (1986) found a biphasic reaction to thermal inactivation of aqueous solutions of purified *Phaseolus* agglutinin (PHA) at 82°C. Based on the calculation of rate constants from their studies and on the study of Grant *et al.* (1982), they indicated that lectins in whole beans are largely protected from thermal inactivation as compared to purified lectins (Boufassa *et al.*, 1986).

The reaction rate computed for TIA inactivation (Figure 2) was found to be biphasic which has also been observed in whole soyabeans (Collins and Beaty, 1980; Liener and Thomlinson, 1981). For *Phaseolus* bean flour, a single first-order reaction was found for the thermal inactivation of TIA at temperatures below 100°C (Buera *et al.*, 1984). Inhibitors from *Phaseolus* are reported to have multiple forms (Tsukamoto *et al.*, 1983). This observation cannot be explained by a biphasic inactivation of a single inhibitor but may be explained by the different inactivation behaviour of several inhibitors which differ in their heat sensitivity. The rapid initial inactivation of TIA may be explained by the catalytic effect of water on the inhibitor protein molecule while during the second phase of heating the denaturation of protein is accomplished mainly by a thermal effect (Roa *et al.*, 1989). Since we used a batch containing several types of beans and the assay for TIA does not discriminate between different functional inhibitors, the results of this experiment do not give an unambiguous explanation.

The PDI value is the percentage of protein being dispersed in water (25°C) after 10 min. Heat treatment causes denaturation of protein with a subsequent decrease in their dispersibility in water. Protein dispersibility data, therefore, provide an indication of the degree of protein denaturation after heating. The heating conditions for whole beans in the present study were observed to cause a decrease in PDI value to a minimum level of 15.1%. This decrease is greatly affected by temperature change (Figure 4) which, at the moisture levels used, is similar to the loss of protein solubility in heated bean flour (Pilosof *et al.*, 1981). The increase in PDI with longer processing times at higher temperatures may be attributed to the hydrolysis of protein. However, we did not find experimental evidence for this phenomenon. 'ALV', as distinct from 'total'

lysine is used to differentiate between lysine which is damaged or modified during protein denaturation and lysine which remains nutritionally available to metabolic processes. The present data indicate that only a small amount of total lysine (TL) was lost on heating for long periods at higher temperatures but that ALV was depressed to a greater extent. This has also been observed for groundnut meal (Roach *et al.*, 1967). The ALV may therefore be of greater value as an indicator of heat damage then total lysine.

Conclusions

The reaction rate constants for ANF were higher compared to those of lysine parameters for steam processing of beans. The effect, however, of excessive processing time on ALV, especially at higher temperatures, emphasize the need for establishing minimum destruction rates of ANF in processed beans which may not exert negative effects when these beans are used for feeding to monogastric animals.

From the results of the present experiment it was concluded that:

1. Steam treatment of beans at 102°C inactivated proteinaceous anti-nutritional factors but is not an economic method due to the long processing times required.
2. Steam treatment at 119°C for 5 or 10 min. seemed to be a good compromise between inactivation of ANF and protein damage as measured by AVL.
3. Further research requires to be carried out to evaluate the effects of steaming at 136°C for <90 seconds.
4. Optimizing the steam process for raw materials to be used in animal feeding must be done in conjunction with *in vivo* experiments on animals.

Bioassay are needed to justify the provisional conclusions from the present investigation and to establish quality parameters after the processing of beans. Research into the effect of steam processing of beans on the apparent digestibility *in vivo* is currently being undertaken.

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CHAPTER V

Steam processing methods for *Phaseolus vulgaris* beans - implications for ileal digestibility of nitrogen and lysine in piglets

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Abstract

Piglets with an average initial weight of 20 kg, fitted with a T-shaped post-valvular ceacum (PVTC) cannula were used to determine the effect on apparent ileal digestibility of nitrogen and lysine of inclusion in the diet of beans processed under different conditions. In each of two experiments a control diet was used that contained protein from casein and herring meal. In Experiment 1 the experimental treatments consisted of beans, steam heated under atmospheric conditions (102°C) for 20, 40, 60 and 80 min., respectively. Experiment 2 comprised six experimental treatments: beans steam heated at 102°C for 60 min; at 119°C for 5 min; at 136°C for 1.5 min. and inclusion at 10 and 20% into the piglets diets, respectively. Intact animals were used to determine faecal digestibility of nitrogen of the same experimental treatments. In Experiment 1, ileal digestibility of lysine and nitrogen and faecal digestibility of nitrogen were significantly reduced by the inclusion of beans, steam heated at 100°C for 20 min. Longer processing times of beans, heated at 102°C increased nitrogen and lysine digestibility of the bean containing diets in all cases, however, absolute digestibility coefficients of bean diets in the range of the control diet were not obtained.
In Experiment 2, the inclusion of 20% beans, processed at 119°C or 136°C revealed an increased ileal digestibility of nitrogen compared to atmospheric steaming for 60 min., whereas steam processing, at 136°C for only 1.5 min. did restore ileal nitrogen digestibility to the level of the control group. With the exception of the apparent nitrogen digestibility of diets with beans processed at 102°C, none of the ileal or faecal digestibility coefficients of nitrogen and lysine at 20% inclusion level were significantly different from an inclusion of beans at a level of 10%.

Introduction

In seeds of the common bean (*Phaseolus vulgaris* L.) proteins with antinutritional or antiphysiological activity such as lectins and trypsin inhibitors are present. These proteins, unless denaturated, affect the overall utilization of nutrients, particularly proteins. This may lead to large losses of dietary and endogenous protein (Pusztai, 1989). In practice, prior to the inclusion of *Phaseolus* beans into diets for monogastric animals, all beans - even relatively non-toxic ones - are considered potentially toxic and therefore are heat treated. This is done because commercially available seeds cannot always be labelled according to antinutritional properties. Often, their correct botanical name is not known. In addition, contamination by other commercial

samples may occur and, therefore, identification is a problem (Grant *et al.*, 1983).

The activity of lectins and trypsin inhibitors is largely destroyed by appropriate thermal denaturation of these proteins during the processing of whole beans (Grant *et al.*, 1982). For example, steam treatment of whole beans inactivates the antinutritional factors to a large extent as characterized by the assay for lectins, functional lectins and trypsin inhibitory activity (Van der Poel *et al.*, 1990b).

Untreated *Phaseolus* bean protein *per se* has been previously noted to be resistant to digestion resulting in a low digestibility of the bean proteins (Liener, 1979; Antunes and Sgarbieri, 1980). The protein, however, may become more susceptible to proteolysis after heating (Romero and Ryan, 1978; Philips *et al.*, 1981). The possible changes in susceptibility of bean protein itself to proteolysis after different heat processing methods and the subsequent impact on *in vivo* protein digestibility, however, has not yet been clarified.

Beans separately processed using methods applicable in the animal feed industry, were reported to have an apparent faecal protein digestibility of 0.43 for steam heated white beans (Rodriguez and Bayley, 1987) or 0.79 for extruded red beans (Myer and Froseth, 1983). As the ileal digestible nitrogen/amino acids have higher correlations to deposited protein in pigs than faecal digestible values (Sauer and Ozimek, 1986; Dierick *et al.*, 1988) ileal values are good sensors for detecting differences in nitrogen/amino acid digestibility after different processing conditions.

The results presented here estimate the magnitude of different steam treatment procedures which affect the apparent digestibility of beans, included in the diet of piglets. The aims of the experiments were:

- the effects of inclusion of steam treated beans in diets on ileal and faecal nitrogen and lysine digestibility
- the comparison of different steam treatment procedures

Materials and methods

Two experiments were carried out in which the apparent ileal and faecal nitrogen digestibility coefficients of steam heated beans using different processing methods were determined. Beans were steam heated under atmospheric conditions (102°C) during four processing times (Experiment 1) or were subjected to higher processing temperatures for shorter times (HTST-processing; Experiment 2).

Animals

The apparent ileal digestibility was measured in castrated male piglets (crossbreds). The animals had been fitted with a post-valvular T-caecum (PVTC) cannula at a liveweight of about 10 kg. Cannula design and surgical procedure were as described by Van Leeuwen *et al.* (1988). After cannulation the animals were housed individually in appropriate cages and were kept at an ambient temperature of 22°C.

Ileal digestibility was determined using 4 (Exp. 1) and 5 (Exp. 2) animals per treatment with an average liveweight of 18.8±1.2 and 21.5±1.2 kg, respectively. The faecal digestibility was measured using other, non-cannulated piglets (5 animals per treatment) with an average liveweight in Experiments 1 and 2 of 21.5±1.4 kg and 23.5±1.4 kg, respectively.

Diets

Each animal received the control diet during two days. This was followed by a period of four days in which an increasing part of the control diet was exchanged by the experimental diet in which 20% processed beans were included. The percentage of the experimental diets was subsequently raised from 25, 50, 75 to 100%. Thereafter, the animals were accustomed to the experimental diets during 7 days.

Experiment 1 comprised 5 treatments:

(1) 100% control diet;

(2-5) 80% control diet + 20% beans, steam heated at 102°C for 20, 40, 60 and 80 min., respectively.

Experiment 2 comprised 7 treatments:

(1) 100% control diet;

(2-4) 80% control diet + 20% steam heated beans;

(5-7) 90% control diet + 10% steam heated beans.

The beans used in Experiment 2 were steam processed at 102°C for 60 min., at 119°C for 5 min. or at 136°C for 1.5 min. Beans, steam heated at 102°C for 60 min. were evaluated in both Experiments 1 and 2 and the same batch of beans was used. Procedures for steam processing were as described by Van der Poel *et al.* (1990b). The experimental diets are further referred by the bean processing conditions (temperature [°C] and duration [min]) such as 102/20, etc.

A control diet was formulated in which dietary protein was supplied mainly by casein and herring meal. All diets were pelleted without steam addition (pellet diameter: 3 mm) and were supplied at a feeding level of 2.6 times the maintenance requirement. Maintenance was assumed 420 kJ ME BW $kg^{-0.75}$ (ARC, 1981). The formulation of the control diet is shown in Table 1. The analytical data pertaining to the composition of the control and the

experimental diets as well as quality parameters of the processed beans are given in Tables 2 and 3, respectively.

Table 1. Formulation of the control diet (Experiments 1 and 2)

Ingredients (%)			
Maize starch	45.6	$CaCO_3$	0.19
Wheat middlings	5.0	$CaHPO_4.2H_2O$	2.86
Casein	13.1	NaCl	0.30
Herringmeal	5.0	DI-methionine	0.11
Cellulose	2.1	L-threonine	0.06
Soybean oil	2.9	L-tryptophan	0.02
Dextrose	16.0	$NaHCO_3$	1.22
Cane molasses	4.0	$KHCO_3$	0.64
VTM[1]	1.0	Marker Cr_2O_3	0.10

[1]Vitamin, trace mineral premix (mg/kg unless otherwise mentioned) supplied by 1 kg of diet, including:
Magnesium oxide, 2000; cupric sulphate.$5H_2O$, 150; zinc sulphate.H_2O, 150; manganese oxide, 150; ferrous sulphate.$7H_2O$, 200; cobalt sulphate.$7H_2O$, 0.5; sodium selenite.$5H_2O$, 0.3; sodium molybdate.$2H_2O$, 3; boric acid, 3; potassium iodide, 5. Retinol, 9000 IU; cholecalciferol, 1800 IU; α-tocopherol, 30; menadione sodium bisulfite, 5; thiamin HCl, 8; riboflavin, 8; Ca-d-panthotenate, 15; pyridoxine HCl, 4; choline/chlorid, 2000; folic acid, 2; cyanocobalamine, 0.02; biotin, 0.3; L-ascorbic acid, 50; inositol, 100; ƒ-aminobenzoic acid, 2.5; ethoxyquin, 125. The remainder was made up of maize starch.

For the determination of ileal digesta flow, chromium oxide (Cr_2O_3) was added to each diet as an indigestible marker.
Feed was administered with water at a ratio of 1:1. Water was supplied ad libitum. The cannulated animals were fed equal amounts of feed, twice daily, at 08.00 and 20.00 h during the digesta collection period. For the determination of faecal data and during the adaptation periods, animals were fed at 08.00 and 16.00 h.

Collection and measurements
Ileal chyme was collected in plastic pouches 12 hours a day and during 5 days following the adaptation period. In order to inactivate the digestive enzymes 1 ml of Merthiolaat (3%) was added.
Faeces were collected using stoma pouches attached around the anus of the animals. After collection, ileal chyme and faeces were weighed, and frozen (-20°C) until analysis.
Ileal and faecal data were obtained by analysing the digesta samples and the counterpart diets. The apparent digestibility coefficients of dry matter and

nitrogen were determined at the terminal ileum and at the end of the digestive tract. Apparent lysine digestibility was determined only at the terminal ileum. Apparent ileal digestion was calculated with reference to the Cr-concentrations of the diets and ileal digesta. The apparent digestibilities of the processed beans were calculated from the difference in digestibility of the control and experimental diets, respectively.

Table 2. Proximate analysis[1] (% DM) of the control and experimental diets (Experiment 1) and levels of lectins, TIA and protein quality of processed beans[2]

	Control	Experimental diets[3]			
		102/20	102/40	102/60	102/80
Diets					
Nitrogen	3.09	3.32	3.34	3.32	3.32
Crude fat	3.9	3.3	3.3	3.3	3.3
Crude fibre	1.9	2.4	2.5	2.4	2.4
Ash	6.5	6.1	6.0	6.1	6.0
Beans					
TIA (mg/g)	11.0	1.50	1.03	0.72	0.45
ELISA (mg/g)	58.0	10.6	3.13	0.72	0.64
ALV (g/16g N)	5.36	5.37	5.28	ND	5.04
PDI (%)	34.2	20.1	18.6	17.6	17.4

[1]After pelleting; means (n = 2).
[2]Control column refers to unprocessed beans; for analytical methods see Van der Poel *et al.* (1990b). ND = not determined.
[3]These diets were formulated to contain 80% of the control diet and 20% of heat processed beans.

Analytical and statistical procedures

Fresh samples of excreta were analysed in duplicate for dry matter and nitrogen (Kjeldahl) according to standard methods. Samples of the excreta were freeze-dried and, along with the samples of the diets, ground and analysed for dry matter, lysine and chromium (Cr; atomic spectrophotometry; Perkin Elmer 300). Lysine values of diets, beans and ileal chyme were determined in duplicate using ion-exchange chromatography (Biotronic LC 2000). Trypsin inhibitor activity (TIA; mg inhibited trypsin/g product), lectins (ELISA; mg/g product), available lysine value (ALV; g/16g N) and protein dispersibility index (PDI; %) were analysed according to methods described previously (Van der Poel *et al.*, 1990b). Data were analyzed with one way analysis of variance (Student Newman Keuls Test).

Table 3. Proximate analysis (% DM) of the control and experimental diets (Experiment 2) and levels of lectins, TIA and protein quality of (un)processed beans[1].

	Control	Experimental diets		
		102/60	119/5	136/1.5
Diets				
Nitrogen	2.97	3.23	3.19	3.20
Crude fibre	2.2	2.4	2.4	2.4
Crude fat	3.6	3.5	3.5	3.5
Ash	6.5	6.2	6.2	6.2
Beans				
TIA (mg/g)	11.0	0.72	0.69	0.62
ELISA (mg/g)	58.0	0.72	0.79	0.08
ALV (g/16g N)	5.36	ND	5.09	4.68
PDI (%)	34.2	17.6	17.3	15.2

[1]See notes Table 2; Means of 2 (Beans and control diet) or 4 (Experimental diets) analysis.

Results

In the Experiments no feed refusals were encountered. The average values for apparent nutrient digestibility of the diets in Exp. 1 are presented in Table 4. The digestibility coefficients of dry matter and nitrogen showed a large and significant ($P<0.05$) difference between the control diet and all experimental diets, particularly the ileal nitrogen digestibility coefficient of the diet with 20 min. heated beans. The inclusion of beans heated for 60 min. or longer did not further increase digestibility of dry matter and nitrogen. For faecal digestibility data, however, nitrogen showed a higher value ($P<0.05$) for the diet of which the beans were heated for 80 min. The ileal digestibility coefficients for lysine follow a similar pattern to those of nitrogen but at a higher level.

The average values for apparent digestibility of diets used in Exp. 2 are presented in Table 5. The digestibility coefficients of dry matter for the control diet used in both experiments are comparable; for nitrogen and lysine the digestibility coefficients were somewhat lower in Exp. 1 than in Exp. 2. The ileal digestibility of dry matter and nitrogen showed large differences between the diets at 20% inclusion. Steaming of beans showed consistent differences ($P<0.05$) for the digestibility values with respect to the

temperature/time pattern of heating. Heating at 102°C or at 119°C did affect the ileal digestibility of dry matter and nitrogen significantly ($P<0.05$) as compared to the control diet. Ileal digestibility was not improved further ($P>0.05$) when beans heated at 136°C were included in the diet. For ileal lysine digestibility a significant decrease was observed for the 102/60 and 119/5 diets compared to the control diet. Similar to nitrogen digestibility, the ileal digestibility of lysine of the 136/1.5 diet was similar to that of the control diet ($P<0.05$).

Table 4. Apparent ileal and faecal digestibility coefficients of dry matter, nitrogen and lysine of diets: effect of atmospheric steaming conditions (Experiment 1)[1].

	Apparent digestibility				
	Ileal			Faecal	
	Dry matter	Nitrogen	Lysine	Dry matter	Nitrogen
Control diet	86.7[a] 0.7	85.4[a] 1.1	91.9[a] 0.9	91.9[a] 1.1	93.4[a] 1.2
Bean diets					
102°C/20 min.	75.7[c] 1.3	69.2[c] 1.7	79.0[c] 1.9	88.8[c] 0.9	82.2[c] 1.0
102°C/40 min.	78.7[b] 1.1	73.5[b] 1.3	82.0[b] 1.8	89.4[bc] 1.1	83.1[c] 1.6
102°C/60 min.	78.4[b] 1.5	74.5[b] 1.6	81.6[bc] 1.5	89.4[bc] 0.7	83.5[c] 0.9
102°C/80 min.	78.4[b] 1.0	76.0[b] 1.4	83.4[b] 1.3	90.4[b] 0.8	85.4[b] 1.2
LSD	1.76	2.66	4.49	1.12	1.76

[1]Means and standard deviation; LSD = least significant difference
[2]Diets containing 20% beans heated at 102°C for different times (min.)
[a,b,c]Data with different superscripts in the same column differ significantly ($P<0.05$)

For ileal digestibility, the coefficients for dry matter and nitrogen of the diets showed a similar tendency with respect to the different bean treatments. Apparent faecal digestibility of nitrogen, however, was reduced significantly ($P<0.05$) for all treatments compared to that of the control diet.
At 10% inclusion, higher ileal and faecal digestibility values of diets are

observed as well as a similar ranking in relation to bean treatment procedures. For the 102/60 bean diet, the ileal and faecal digestibility of nitrogen and the ileal digestibility of lysine was significantly lower than those of the control diet.

Table 5. Apparent ileal and faecal digestibility coefficients of dry matter, nitrogen and lysine of diets: effect of different steam processing methods and bean inclusion level (Experiment 2)[1]

	Apparent digestibility				
	Ileal			Faecal	
	Dry matter	Nitrogen	Lysine	Dry matter	Nitrogen
Control diet	86.2[a]	81.7[a]	86.8[a]	92.7[a]	93.5[a]
	1.8	2.1	1.6	1.3	1.4
Bean diets					
20% inclusion level					
102°C/60 min.	76.3[d]	70.0[d]	78.4[c]	90.2[b]	84.7[d]
	2.1	2.6	2.5	1.3	2.0
119°C/ 5 min.	80.1[cd]	76.9[bc]	81.9[b]	91.4[ab]	89.5[c]
	1.5	1.9	2.0	1.0	0.8
136°C/1.5 min.	83.5[ab]	80.9[ab]	84.6[ab]	91.9[ab]	90.8[bc]
	2.0	2.3	2.4	1.3	2.0
10% inclusion level					
102°C/60 min.	81.0[bcd]	75.1[c]	81.5[b]	91.1[ab]	88.7[c]
	1.0	1.2	1.2	1.0	1.2
119°C/ 5 min.	81.9[bcd]	78.0[abc]	82.9[ab]	91.5[ab]	90.0[bc]
	1.1	1.6	1.5	0.9	1.2
136°C/1.5 min.	82.4[bc]	79.6[abc]	84.4[ab]	92.3[a]	92.3[ab]
	1.5	1.7	1.9	0.8	0.6
LSD	3.24	4.55	3.95	1.76	2.56

[1]Means and standard deviation; LSD = least significant difference
[a,b,c,d]Data with different superscripts in the same row differ significantly ($P<0.05$).

Digestibility coefficients of the processed *Phaseolus* beans itself (20% inclusion level) were calculated from the data of Tables 4 and 5 and presented in Figure 1 for nitrogen and lysine digestibility coefficients. A clear relationship between apparent ileal digestibility in pigs and bean processing can be derived

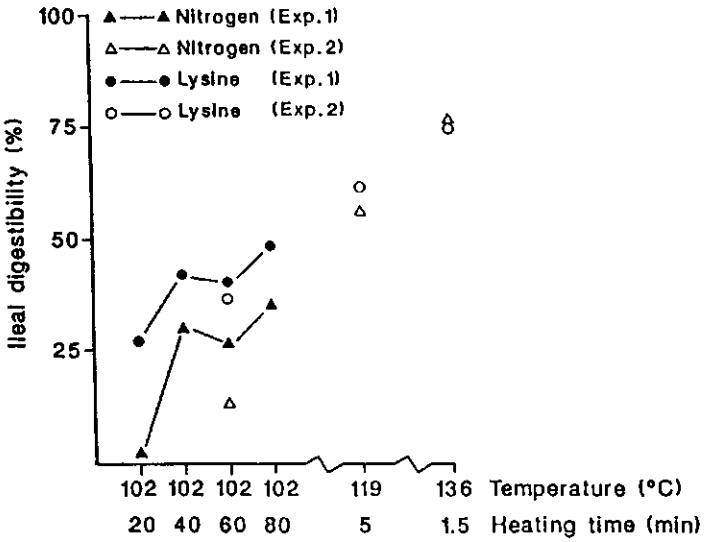

Figure 1. Apparent ileal digestibility of nitrogen and lysine of heat processed beans (Indirect method).

in that short steam processing times at 119°C and 136°C (HTST-processing) establish a distinct improvement of apparent ileal digestibility of nitrogen and lysine from beans compared to 102°C/20-80 min. (atmospheric steaming). The dry matter content of the ileal chyme was only significantly affected by the dietary treatments 100/20 and 100/80 in Exp. 1 (Table 6) but was not affected by the treatments in Exp. 2. However, the dry matter content of ileal chyme from animals fed the control diets used in Exp. 2 was lower compared to that of Exp. 1. The ileal digestibility of the control diet was similar in both experiments. Thus, the higher amount of chyme in Exp. 2 was compensated by a lower dry matter content. In correspondence with the digestibility values, total ileal chyme produced per kg of feed intake was increased after the inclusion of steam heated beans. Steaming for 80 min (102°C) still showed a significant increase for ileal chyme excretion but HTST-processing (Exp. 2) showed similar ileal chyme excretion than the control diet. Heat treatment of beans at different conditions did not significantly ($P>0.05$) influence the dry matter content of the faeces, however, in general dry matter content of the faeces was lower than that of the control animals. The amount of faeces produced per unit of feed intake was not greatly influenced by the several bean treatments used in Experiments 1 and 2, but was significantly ($P<0.05$) increased in comparison with the control diet.

Table 6. Dry matter content and excretion of ileal chyme and faeces[1,2].

	Dry matter (%)		Excretion (g/100g feed)	
	Ileal chyme	Faeces	Ileal chyme	Faeces
Experiment 1				
Control diet	$13.7^{a} \pm 1.3$	$42.7^{a} \pm 2.1$	$87.8^{c} \pm 3.7$	$17.4^{c} \pm 2.1$
Bean diets				
102°C/20 min.	$9.0^{c} \pm 1.0$	$36.4^{b} \pm 2.1$	$243.5^{a} \pm 5.8$	$27.9^{a} \pm 2.0$
102°C/40 min.	$10.1^{bc} \pm 0.7$	$33.9^{b} \pm 1.7$	$187.0^{b} \pm 2.7$	$27.9^{a} \pm 1.6$
102°C/60 min.	$10.2^{bc} \pm 0.7$	$39.5^{ab} \pm 2.3$	$189.7^{b} \pm 5.0$	$24.4^{ab} \pm 1.9$
102°C/80 min.	$11.4^{b} \pm 1.3$	$37.8^{ab} \pm 2.0$	$171.8^{b} \pm 5.5$	$23.2^{b} \pm 1.5$
LSD	1.58	5.95	30.3	4.0
Experiment 2				
Control diet	$11.4^{a} \pm 1.6$	$42.7^{a} \pm 1.9$	$115.6^{b} \pm 6.6$	$15.7^{b} \pm 2.0$
Bean diets				
102°C/60 min.	$10.0^{a} \pm 1.0$	$35.2^{b} \pm 2.8$	$217.3^{a} \pm 7.2$	$26.4^{a} \pm 3.1$
119°C/ 5 min.	$11.3^{a} \pm 0.9$	$35.2^{ab} \pm 2.8$	$159.4^{b} \pm 5.0$	$19.8^{b} \pm 1.6$
136°C/1.5 min.	$12.1^{a} \pm 1.6$	$36.5^{b} \pm 2.4$	$129.2^{b} \pm 7.4$	$20.7^{ab} \pm 2.7$
LSD	2.53	5.19	49.3	6.0

[1]Means and standard deviation.
[2]20% bean inclusion level.
[a,b,c]Data with different superscripts in the same column are significantly different ($P<0.05$).

Discussion

The average values for the faecal and ileal digestibility coefficients of dry matter and nitrogen for the control diet were in agreement with earlier observations for diets with similar composition (Huisman *et al.*, 1990; Van der Poel *et al.*, 1990a). The variation between animals within diets for faecal digestibility was at a normal level; for ileal values the variation was relatively low.

The apparent digestibility of nitrogen in the diets based on casein, herring meal and beans increased with heating time of beans (102°C; Exp. 1). This is in agreement with the results of Rodriguez and Bayley (1987) and of Van der Poel *et al.* (1990a) on apparent faecal digestibility for the piglets of average weights of 36.3 and 8.3 kg, respectively. In the present investigation, diet digestibilities were higher than those observed by Rodriguez and Bayley

(1987). This can be explained by the different composition of the control diets in our study and in the latter study. The protein sources, in the latter study in addition to beans, were soyabeans and maize.

In Experiment 1 different processing times for beans, steamed at 102°C resulted in various residual levels of lectins and of trypsin inhibitor activities. Beans, heated at 102°C for only 20 min. still showed substantial residual TIA (15%) and lectin (ELISA) levels (20%) relative to unprocessed beans. The inclusion of these beans lead to a significant ($P<0.05$) decrease in ileal digestibility of nitrogen and of lysine. It is further noted, that this result agrees with the observation that this dietary treatment (underprocessing of beans) caused a visibly lower health status of the animals involved as shown by a less shining hair coat and long hair. In addition, those animals were irritated by the change of pouches during the collection of ileal chyme. In the animals fed other dietary treatments this was not observed.

The range of processing conditions as studied in Experiment 2 appeared to be adequate as characterized by the residual levels of lectins and trypsin inhibitory activity in the processed beans. According to Table 3 the antinutritional factors have been eliminated after heating at all conditions. In this experiment, there was a large difference between diets in ileal digestibility coefficients of lysine and of nitrogen in particular and, therefore, also between the bean proteins. A short exposure of beans to high temperature processing will affect the apparent digestibility of bean protein more favourably than atmospheric steaming at 102°C (Table 5; Figure 1). Furthermore, the residual levels of ANF in beans after steaming at 102°C for 40 or 60 min. did not significantly affect ileal digestibility coefficients of beans compared to the control diets. On this basis it can be derived that the residual levels of both lectins and trypsin inhibitor activity in beans after steaming under the conditions of Experiment 2 cannot be a very significant cause for the relative large differences in ileal apparent nitrogen digestibility observed for these beans. Moreover, it should be noted, that from the total dietary protein only a part is derived from bean.

It has been suggested that the existence of structural constraints for digestive enzyme hydrolysis of the major storage proteins of *Phaseolus vulgaris* seeds, Glycoprotein II (Romero and Ryan, 1978; Liener and Thompson, 1980) may result in incomplete hydrolysis to amino acids. This may therefore limit bean protein utilization in itself, even in the absence of toxic bean lectins. During thermal activation, the conformation of the protein is altered, generally termed "denaturation" (Lapanje, 1978; Grinberg *et al.*, 1989). Denaturation of proteins will cause an inactivation of proteinaceous ANF. Initially it may improve digestibility, but severe denaturation may decrease digestibility through protein-protein crosslinking, protein-carbohydrate crosslinking or otherwise.

Denaturation is brought about by a combination of moisture content, temperature, time and the presence of other interfering constituents such as for instance carbohydrates. Severe processing has been reported to cause several types of linkages between denaturated molecules. It is thought that this leads to protein aggregation (Bacon *et al.*, 1989) and insolubility (Sheard *et al.*, 1986) at elevated temperatures. Research efforts towards the influence of water content, temperature and time, however, have been mainly studied in systems using protein isolates or protein concentrates from beans at high moisture levels. The relevance of these studies to those in which bean are thermally processed at higher temperatures and at lower moisture levels is difficult to evaluate. For example, the temperature needed to denaturate soya protein has been observed to be more dependent on the water content of the system in the flour than in the protein isolate (Sheard *et al.*, 1986). This suggests that carbohydrates-water or carbohydrate-protein interactions may play a role in the denaturation process. In addition, a multiplicity of chemical phenomena may occur in systems containing proteins as well as carbohydrates under "adverse" thermal conditions, f.e. the so-called browning reaction.
The observed loss of nitrogen dispersibility (PDI) after heating of beans (Van der Poel *et al.*, 1990b) suggests that some conformational and/or aggregational changes may have taken place. For PDI loss, however, differences between treatments were relatively small.
Sheard *et al.* (1986) pointed out that some conformational changes take place after prolonged heating of proteins at temperatures below which normally leads to denaturation. The impact of this phenomena on proteolysis has not yet been fully established. Denaturation of Glycoprotein II takes place at about 95°C (Wright and Boulter, 1980). Heating the Glycoprotein II at 100°C for only 10 min. did not change the extent or the rate of proteolysis by sequential treatments with stomach and small intestinal extracts of rats compared with native Glycoprotein II (Sgarbieri *et al.*, 1982). Prolonged heating (60 min at 100°C) of *Phaseolus* bean protein concentrates, however, suggest an aggregation of the major storage proteins, thus increasing their resistance to *in vitro* proteolysis (Deshpande *et al.*, 1983).
In general, heat may render lysine unavailable, an effect which, obviously, becomes crucial only at severe degrees of overheating (Geervani and Theophilus, 1980; Hang *et al.*, 1980). The apparent digestibility coefficients of lysine in Experiments 1 and 2 indicate that the heat treatments used were not excessive for total lysine.
Prolonged heating, although effective in lowering the residual levels of proteinaceous ANF, may have an adverse effect on conformation of the protein and on subsequent proteolysis. This effect probably cannot take place at conditions of a short exposure to steaming during HTST-processing.

Optimization of the HTST-processing of beans, therefore, requires further elucidation of conformational changes of proteins at the molecular level.

The results of the present investigation show that:

- The elimination of lectins and trypsin inhibitor activity can be obtained by atmospheric steaming (102°C) during long processing times. These treatments are useful as characterized by their residual levels. However, processing times which exceeded 40 min. do not further increase the bean ileal apparent digestibility of nitrogen, contrary to lysine.
- For beans in which ANF have been eliminated nearly completely by different processing methods, the highest ileal nitrogen digestibility coefficient was measured in beans subjected to HTST-processing. The digestibility coefficients of nitrogen and lysine from beans were increased but only steaming at 136°C for 1.5 min. restored ileal protein digestibility to that of the control group in which casein and herring meal were the main protein sources.
- The physico-chemical properties of protein, heated under HTST-conditions and consequences for proteolysis, require further investigation.

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CHAPTER VI

Evaluation of Techniques to determine the Protein Digestibility in Heat-Processed Beans for Piglets

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Abstract

Studies have been made with four (30 to 45 kg liveweight) barrows to evaluate the mobile nylon bag technique (MNBT) and two *in vitro*enzymatic methods for determining and to predict the apparent ileal dry matter (DM) and nitrogen (N) digestibility in a variety of heat treated bean samples (*Phaseolus vulgaris* L.). For MNBT estimates, one gram samples of beans, steam heated under different time/temperature conditions were enclosed in nylon bags. After predigestion (pepsin-HCl) and *in vivo* digestion (duodenum to terminal ileum) by piglets, the undigested contents of ten bags per bean treatment were pooled within pig and sampled prior to analysis. The disappearance of DM and N of samples were also determined with four pigs of a liveweight of 70-80 kg. In addition, the effect of two basal diets was examined.

In vitro values of digestibility were determined using either a multi-enzyme pH-stat assay or a multi-enzyme N-digestibility assay. With the MNBT, the digestibility of DM or N did not differ between weight classes of pigs and also, no differences were observed between the basal diets used. The apparent ileal digestibility values in unprocessed beans were high, ~78% (DM) and ~92% (N). The MNBT showed a clear trend in digestibility values in relation with bean processing methods.

The used *in vitro* enzymatic procedures showed no acceptable estimates for absolute values or ranking of N and DM digestibility. For the digestibility of N ($P<0.01$) and DM ($P<0.05$) significant correlations were found between MNBT ileal digestibility and *in vivo* ileal digestibility, determined with the same samples in a previous experiment.

Introduction

The supply of major nutrients is studied in digestibility trials. Conventional methods to measure digestibility involving total collection of ileal chyme or faeces do have some limitations. They require large amounts of feed and a relatively long period is needed for adaptation to the experimental diets (Pedersen and Eggum, 1983; Cherian *et al.*, 1989). These methods, therefore, are costly and time consuming. Much research has been devoted to the development of more rapid techniques including *in vitro* digestibility procedures to overcome the inherent shortcomings of these conventional methods. In addition to several *in vitro* digestibility assays (see Moughan *et al.*, 1989) a Mobile Nylon Bag Technique (MNBT) has been proposed (Sauer *et al.*, 1983).

The technique for studying nutrient digestibility by oral administration of nylon bags (Petry and Handloss, 1978) was further adjusted to pigs fitted with a single cannula in the duodenum for measuring faecal MNBT digestibility (Sauer *et al.*, 1983) or ileal digestibility (Van Leeuwen *et al.*, 1988, unpublished results). The MNBT allows a rapid measurement of ileal nutrient digestibility in the intestinal tract and is considered a more practical method for routine and rapid evaluation of complete diets or of single diet ingredients. Especially for dietary ingredients of low palatability such as beans, the MNBT offers a new way for determining or predicting nutrient digestibility. Validation of the MNBT with the conventional methods (CM) for determining apparent digestibility coefficients, however, is needed. For a number of feedstuffs, the use of the MNBT for predicting faecal protein digestibility for pigs has been evaluated (Cherian, 1985; Cherian *et al.*, 1988; Sauer *et al.*, 1989). Nutrient digestibility as measured by total collection or by MNBT may differ for ANF containing feedstuffs or in feedstuffs, heat processed at different conditions (Huisman *et al.*, 1988).

The aim of the present studies was to evaluate the use of the MNBT as a means to predict the ranking of nitrogen and dry matter digestion of bean (*Phaseolus*) samples passed through the intestinal tract in nylon bags and recovered at the terminal ileum. Bean samples were steam heated under different conditions. In addition, two multi-enzyme techniques were used for *in vitro* values. Comparison with *in vivo* data obtained by total ileal chyme collection was made.

Materials and methods

Experimental design

A series of two experiments were conducted to asses the effect of different steam heating procedures on the digestibility (referred to as 'apparent') of dry matter and nitrogen in the small intestine using MNBT.

The effect of heating beans was examined using MNBT in pigs of two weight classes. Beans which were atmospheric steamed (102°C) during different processing times were used. In addition, the effect of two basal diets was examined using unprocessed beans and using beans processed at different time/temperature conditions.

The effect of different steam treatments was further assessed using two *in vitro* procedures based on incubation with different enzymes.

Values of protein digestibility estimates by these two methods were compared with *in vivo* data. *In vivo* digestibility coefficients were determined in digestion trials using 4 or 5 animals per treatment (Van der Poel *et al.*, 1990a) at a

feeding level of 2.6M. (M is the metabolizable energy (ME) requirement for maintenance as a function of liveweight (LW) = 420 kJ $LW^{-0.75}$).

Animals and diets

Four barrows (Dutch Landrace x Great Yorkshire) were fitted surgically with a simple duodenal T-cannula (Sauer *et al.*, 1983) and a post-valvular T-caecum (PVTC) cannula at the terminal ileum (Van Leeuwen *et al.*, 1988). After surgery, the piglets were placed immediately in metabolism cages and fasted that day. The next day, they were given approximately 100 g of a commercial starter diet. This was followed by a period of 9 days in which an increasing part of the commercial diet was exchanged by a basal (bean) diet (BBD) in which 20% heat processed beans (102°C during 60 min) were included. The percentage of the experimental diet was set at 25, 50, 75 and 100%. Following a period of eight days recovery to normal appetite, feed allowance was 2.6 times the ME requirement for maintenance (420 kJ $LW^{-0.75}$) as specified by ARC (1981). Water was administered with the feed at a ratio of 2:1. In addition, water was freely available through drinking nipples. Following the same procedure digestibility was also determined in older pigs. Four barrows were used of the same crossbred with a liveweight of 70-80 kg. In addition, to the basal bean diet (BBD), a standard-type basal diet (SBD) was examined, generally used in MNB-studies at the TNO-ILOB Institute in Wageningen. The formulation of the basal diets as well as the analysed contents of dry matter and nitrogen are shown in Table 1.

Table 1. Formulation of the basal diets

Bean diet		Standard diet	
Beans[1]	20.0	Barley	83.4
Casein	13.1	Soybean meal (46% CP)	12.0
Herring meal	5.0	VTM[3]	1.0
Maize starch	45.5	Minerals[3]	3.6
Dextrose	16.0		
Wheat middlings	5.0		
Cellulose	2.1		
Soybean oil	2.9		
Molasses	4.0		
Dl-methionine	0.11		
L-threonine	0.06		
L-tryptophan	0.02		
VTM[2]	6.20		

[1]Refers to beans, steam heated for 60 min.
[2]For Vitamin and (Trace)Mineral composition, see Van der Poel *et al.* (1990a).
[3]For the composition of the Vitamin (Trace)Mineral and mineral mixture, see Van Leeuwen *et al.* (1987).

Procedure MNBT

Monofilament nylon bags (25x40 mm; pore size 40 μm) were prepared as described by Sauer *et al.* (1989), filled with a 1-g finely ground (1 mm) bean sample and were sealed. Bean samples with known apparent ileal and faecal digestibility of nitrogen and dry matter were used, previously determined by total collection of ileal chyme or faeces (difference method) with pigs weighing about 20 kg were as described by Van der Poel *et al.* (1990a).

Prior to insertion into the duodenum of the piglets the bags containing bean samples were predigested to simulate stomach digestion. Ten bags at a time were immersed in a beaker containing 500 ml of a solution made up of deionized water, 0.01 *N* HCl and purified pepsin powder (Merck, 7185; 0.8 g pepsin/500 ml solution). The beaker was placed in a waterbath of 37°C, agitated (90 oscillations/min) for 5 hours. Afterwards, bags were frozen until required.

Initiating the experiments, frozen bags were thawed (waterbath; 37°C for 5 min). Ten bags per bean treatment were prepared for each pig. Two bags were inserted each hour during a 5-min period at 08.00, 09.00, 10.00, 11.00 and 12.00 h, respectively. Bags were retrieved via the PVTC-cannula during 12 hours after the first insertion. Collected bags were carefully isolated, coded and frozen immediately.

Procedure in vitro enzymatic assays

Procedure 1. The *in vitro* digestibility of protein (IVPD) was assessed by analysing 7 samples of heat processed beans in duplicate using a modified Procedure A as described by Babinszki *et al.* (1990). In this procedure, sample materials are incubated with pepsin/hydrochloric acid solution at 40°C for 1.5 h. After neutralization ($NaHCO_3$) incubation is continued (40°C; 1 h) with potassium phosphate buffer, containing hog pancreatine, α-amylase, lipase and bile salts. Following incubation, Na_2CO_3 is added to stop the reaction. The undissolved material is then filtered in a glass filter crucible fitted with a layer of N-free ashless floc. The undigested residue and the ashless floc is brought into a Kjeldahl flask for the determination of the N content by the Kjeldahl method. The digestibility coefficient is calculated by difference from the N content of the bean sample before digestion.

Procedure 2. In a second *in vitro* procedure, the three-enzyme system (Hsu *et al.*, 1977) was applied to the same bean samples and Na-caseinate in a pH-stat procedure as described by Pedersen and Eggum (1983). Samples were pretreated with NaOH and the pH was adjusted to 8.00 and kept constant (automatic titration; titrant NaOH) during the incubation with enzymes (trypsin, α-chymotrypsin and peptidase). At the end of 10 min. incubation, the amount of alkali added was recorded and this value was used for estimation

of *in vitro* protein digestibility, relative to Na-caseinate.

Analytical and statistical procedure

Following thawing and washing with water, bags from the MNBT experiments were freeze-dried, pooled within pig and bean treatment and analyzed for N using the Kjeldahl procedure (ISO, 1979). Dry matter was determined using a standard method. Proximate analysis of beans and analysis of the levels of trypsin inhibitor activity and lectins present in un(processed) beans were carried out as described by Van der Poel *et al.* (1990b).

Treatment means were tested for significance ($P<0.05$) using Tukey's procedure. Mean digestibility coefficients of CM (7 values were used; see Van der Poel *et al.*, 1990a) were determined) were subjected to a one-way regression analysis using the following model: $Y_{ij} = \mu + b * T_i + e_{ij}$ where T_i = treatment mean for MNBT, pH-stat or IVPD (SAS-Reg, SAS 1985).

Results

The mean recovery of bags in these studies was about 90%. Bags were recovered within 9 h after the insertion of the first bag at 08.00 h.

The data pertaining to the analysed composition of the steam heated beans and the basal diets used in the MNBT experiment are shown in Table 2. The standard diet used to assess the digestibility in bean samples was included in the experiment to compare absolute digestibility values of other feedstuffs evaluated in previous experiments using the MNBT.

Table 2. Proximate composition (% DM) of the two basal diets and beans.

	Basal diets		Beans
	Bean diet	Standard diet	
Nitrogen	3.34 ± 0.1	2.82	3.82
Crude fat	3.3 ± 0.2	2.2	1.7
Crude fibre	2.3 ± 0.1	4.4	3.5
Ash	6.1 ± 0.1	6.8	3.9
TIA	0.72	--	DHT[1]
Lectins (ELISA)	0.72	--	DHT

[1]DHT, Depending on Heat Treatment (see Van der Poel *et al.*, 1990b)

Four bean samples, heated at 102°C for 20, 40, 60 and 80 min., respectively, were studied in the two weight classes of pigs. The data on disappearance of dry matter (DM) and nitrogen (N), as determined with the MNBT showed slight differences between the examined weight classes of pigs (Table 3). A similar ranking of apparent DM and N digestibility coefficients was observed between weight classes, although the digestibility did not follow a consecutive order depending on processing time of the beans.

Table 3. Apparent nitrogen and dry matter ileal digestibility[1] (%) of bean samples steamed for various duration at 102°C in pigs of two weight classes (determined with MNB Technique).

	30-45 kg		70-80 kg	
	Dry matter	Nitrogen	Dry matter	Nitrogen
102°C/20 min.	74.6 ± 0.5	89.6 ± 0.2	74.8 ± 0.7	89.0 ± 0.7
102°C/40 min.	78.8 ± 0.2	92.2 ± 0.3	79.2 ± 0.6	92.0 ± 0.5
102°C/60 min.	76.2[b] ± 0.5	91.3 ± 0.7	77.5[a] ± 0.6	91.3 ± 0.5
102°C/80 min.	76.7 ± 0.6	91.9[a] ± 0.4	76.9 ± 1.0	91.0[b] ± 0.8

[1]Means (n=4) and standard deviation; LSD_{DM} = 1.29, LSD_{N} = 0.94.
[2]Data with a different superscript in a row differ significantly (P<0.05).

The apparent digestibility values for unprocessed beans in young piglets were relatively high and about 78% (DM) and 92% (N) (Table 4).
The results for the influence of the type of basal diet are summarized in Table 4. No differences (P>0.05) were observed for the calculated DM/N digestibilities between the basal diets. For both diets, however, there is a clear trend in digestibility values in relation to bean processing methods, associated with MNBT. Atmospheric steaming of beans for 60 min and 80 min did show apparent digestibility coefficients which were consistently lower compared to 40 min heating.
The apparent MNBT estimates of DM and N digestibility along with *in vivo* estimates (conventional method) and *in vitro* enzymatic values for N digestibility are given in Table 5. Overall, the results of the determination of MNBT digestibility demonstrate significant differences between bean samples processed at different thermal conditions. The *in vitro* estimates of nitrogen digestibility by pH-stat and IVPD show more variable results than MNBT values.

Table 4. Effect of different steam treatments of beans on dry matter and nitrogen ileal digestibility[1] in pigs (30-45 kg) as measured with MNBT at two basal diets.

	Bean diet		Standard diet	
	Dry matter	Nitrogen	Dry matter	Nitrogen
unprocessed	78.2[a] ± 0.4	91.7[a] ± 0.2	78.4 ± 0.6	91.5 ± 0.3
102°C/20 min.	74.6[c] ± 0.5	89.6[b] ± 0.2	74.7 ± 0.7	90.4 ± 0.7
102°C/40 min.	78.8[a] ± 0.2	92.2[a] ± 0.3	78.9 ± 0.6	93.3 ± 0.6
102°C/60 min.	76.2[b] ± 0.5	91.3[a] ± 0.7	77.0 ± 0.7	91.9 ± 0.4
102°C/80 min.	76.7[b] ± 0.6	91.9[a] ± 0.4	77.0 ± 0.7	92.6 ± 0.9
unprocessed	78.2[b] ± 0.4	91.7[b] ± 0.2	ND	ND
119°C/5 min.	79.8[a] ± 0.4	94.2[a] ± 0.6	79.0 ± 0.9	95.0 ± 1.0
unprocessed	78.2[b] ± 0.4	91.7[b] ± 0.2	ND	ND
136°C/1.5 min.	80.0[a] ± 0.6	94.5[a] ± 0.2	79.5 ± 1.1.	94.8 ± 0.9

[1]Means and standard deviation (n=4); ND = not determined
[a,b,c]Means in the same column with a different superscript differ significantly ($P<0.05$).

Table 5. Apparent ileal dry matter and nitrogen digestibility (%)[1] of bean samples determined with the MNBT (bean basal diet), the conventional method (CM)[2] and in vitro digestible estimates of N.

	Dry matter		Nitrogen			
	MNBT	CM	MNBT	CM	pH stat	IVPD
102°C/20 min.	74.6[d]± 0.5	31.8	89.6[c]± 0.2	4.7	70.3	86.1
102°C/40 min.	78.8[b]± 0.2	46.9	92.2[b]± 0.3	31.2	77.1	89.1
102°C/60 min.	76.2[c]± 0.5	45.2	91.3[b]± 0.7	26.3	77.9	87.8
102°C/80 min.	76.7[c]± 0.6	45.5	91.9[b]± 0.4	35.6	80.1	87.0
119°C/5 min.	79.8[ab]± 0.4	55.9	94.2[a]± 0.6	57.6	78.2	86.5
136°C/1.5 min.	80.0[a]± 0.6	72.7	94.5[a]± 0.2	77.7	76.3	85.0

[1]Means and standard deviation (MNBT n=4; CM n=5)
[2]20% bean inclusion level; Two estimates for 60 min. treatment; Data from Van der Poel *et al.* (1990a).
[a,b,c,d]Different superscripts in a column mean significant differences ($P<0.05$).

The results of regression analyses of CM ileal digestibility on various *in vitro* measurements of digestibility are presented in Table 6. There was no significant correlation of pH-stat and IVPD estimates with *in vivo* observations. There was a statistically significant correlation of MNBT with CM values. A high correlation (r = 0.94; P<0.01) was obtained for nitrogen digestibility measured *in vivo* (CM) and with MNBT. This indicates that both the latter assays ranked the N digestibility of the processed beans similarly. For DM a correlation of 0.83 (P<0.05) was calculated.

Table 6. Results of regression of CM[1] apparent ileal digestibility (Y) on various measurements (X).

Product	Technique	n	Coefficients[2]	r	RSD
Dry matter	MNBT	7	-392.4 + 5.68	0.88*	7.06
Nitrogen	MNBT	7	-1263.4 + 14.09	0.96**	7.97
	pH-stat	7	-195.9 + 3.01	0.37	25.76
	IVPD	7	864.0 - 9.52	-0.50	23.94

[1]Conventional method: total ileal chyme collection
[2]Model:Y = a + bX. Y = digestibility coefficient in CM; X = digestibility coefficient in MNBT, pH-stat or IVPD; n = number of samples; r = correlation, RSD = residual standard deviation.
* P<0.05; ** P<0.01

Discussion

Technological treatments generally provide a large number of samples to be tested. It is important therefore, to assess the effects of alternative *in vivo* and *in vitro* digestibility techniques to enable more rapid and less costly techniques. The MNBT was used to asses the digestibility of dry matter and nitrogen in steam processed bean samples, previously evaluated by measuring apparent digestibility by total ileal chyme collection (Van der Poel *et al.*, 1990a). The basal diets used did not influence the digestibility values for N and DM. This is in accordance with earlier results of a MNBT study (Metz *et al.*, 1986, unpublished results). In the latter study, replacement of barley in the standard diet (Table 1) by either 20% dried beet pulp, 5% animal fat or both, did not affect MNBT digestibility.
The apparent protein digestibility determined by the conventional method (CM) differed widely with the steam processing conditions used (Van der Poel *et al.*, 1990a). The measurement made with the MNBT differed much less

between varying processing conditions compared to the CM method (Table 5). In general, estimates of protein digestibility obtained by the MNBT were relatively high. The digestibility of the bean samples could not be adequately predicted (Table 6) by the pH-stat procedure using the three-enzyme technique (Pedersen and Eggum, 1983) or by the IVPD-method using pepsin followed by a.o. pancreatin and α-amylase.

MNBT has been used as a rapid method of assessment of protein digestibility for several groups of feedstuffs either unprocessed or with low contents of ANF (Fentener van Vlissingen *et al.*, 1988; Sauer *et al.*, 1989). However, bean protein digestibility may vary with the level of ANF and the resistance to digestion by proteolytic enzymes of the major storage protein (Liener and Thompson, 1980). Both properties are influenced by thermal treatments, primarily necessary to inactivate lectin proteins present in *Phaseolus* beans. Heat treatment has been proved to inactivate lectins and trypsin inhibitor activity (TIA) (Grant *et al.*, 1982; Van der Poel *et al.*, 1990b). In the present MNBT study, small samples in bags from beans with low levels of residual ANF after heating were used. The MNBT, therefore, is suggested not to allow for the measurement of interactions between the samples investigated (TIA; lectins) and the digestive tract (Huisman *et al.*, 1988; Sauer *et al.*, 1989). In their study (Huisman *et al.*, 1988), ileal MNBT determinations did not predict the negative effect of antinutritional factors in soya and *Phaseolus* beans. In this respect, protein characteristics may contribute to the rate of hydrolysis as suggested by Pederson and Boisen (1982). With these suggestions in mind, it is interesting to note that, with respect to the accessibility for proteolysis of storage proteins, the bean protein properties as measured with MNBT or *in vitro* methods, may serve as an index of protein quality after heating. As mentioned before, the MNBT does not give an accurate prediction of protein digestibility with absolute values for apparent ileal digestibility values (CM) as reference (Table 5). This method, however, has a good discriminating ability between the bean samples. The ranking of the digestibility of nitrogen and dry matter is similar for MNBT and CM. The results obtained by the MNBT, however, were less variable than those observed with the CM.

From Table 5 it is evident that protein digestibility determined using pH-stat and IVPD procedures deviate considerably from the CM values. The protein digestibility of bean samples, heated at 102°C for different times confirms the suggestion that samples may behave differently when assayed by the *in vitro* enzymatic methods. It has been previously noted that these assays may not be sensitive to certain physical and chemical characteristics of materials assayed, such as antinutritional factors (Wolzak *et al.*, 1981). The IVPD method resulted in greater uniformity among the samples and less possibilities to find differences between products. For several feedstuffs including oilseed meals,

however, a high correlation (r=0.99) was found for *in vivo* protein digestibility with this method (Babinszky *et al.*, 1990).

The pH-stat procedure was included in the experiment for enzymatic digestion at a constant pH and has been shown to offer an improved accuracy compared to original enzymatic methods where a pH drop is recorded after incubation (Pedersen and Eggum, 1983). Different protein categories differ in their susceptibility to proteolysis (Pedersen and Eggum, 1983). Based on the present investigations there seems to be some correlation between the pH-stat procedure and *in vivo* digestibility values for beans heated for different times at 102°C. Trypsin inhibitors present in samples may decrease the estimates for digestibility by the pH-stat procedure. This might have been the case with samples, heated at 102°C for 20 min. With longer processing times, lower levels of TIA were obtained (Van der Poel *et al.*, 1990b). However, the levels of TIA in samples, heated at 119°C (5 min.) and of 136°C (1.5 min.) were similar and the pH-stat procedure did not give an accurate prediction of protein digestibility of these samples (Table 6). It is suggested that the rates of bean protein hydrolysis vary with the applied thermal treatments.

In conclusion, the *in vitro* enzymatic procedures used showed no acceptable estimates for absolute values or ranking of N and DM digestibility. The N and DM digestibility of steam treated beans, determined with the MNBT gave values which ranked similarly to those obtained by the conventional digestibility method involving total collection of ileal chyme.

The MNBT using nylon bags of 25 x 40 mm (40 μm pore size) and a sample size of 1 g (1 mm ground) obviously has discriminative ability for ranking N digestibility in beans, heat processed under different conditions. Since a large number of technological treatments can be tested within the same animal, the MNBT appears to be a promising approach for a rapid prediction.

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GENERAL DISCUSSION

Introduction

Antinutritional factors (ANF) such as lectins and protease inhibitors from common beans (*Phaseolus vulgaris* L.) usually interfere with appetite, absorption and metabolism in most species of monogastric animals, unless these beans are processed. Therefore, these factors may play an important role in the nutrition and health of these animals. Especially lectin proteins from beans appear to be highly deleterious (Chapter I).

The feeding of inadequately processed beans or bean fractions may result in a poor performance by the animal. Antinutritional factors affect the overall utilization of nutrients, particularly proteins. Ingested lectins are responsible for damage to the gut mucosa resulting in interference with normal intestinal digestion and/or absorption of nutrients. Another drawback of *Phaseolus* bean protein is that a major proportion is the storage protein, globulin. This protein is very resistant to proteolysis and thus a further reason for the poor utilization of protein by the animal.

Elimination of lectins and protease inhibitors seems possible by genetic manipulation. The desirability of this technique may be questioned because of the proposed roles which these factors play in the defense mechanism of legumes against insect and microbial predation (Liener, 1989). Another way of eliminating ANF is by processing. In the investigations described in this thesis, fractionation of beans and thermal processing were studied as ways to remove or inactivate the levels of ANF. Effectiviness of processing was measured *in vivo*, *in vitro* and by using various chemical assays. As species differences have been observed for the negative effects of feeding processed beans and because plant proteins are often used in diets for pigs, the effects of steam processing were investigated *in vivo* using pigs.
The results of the experiments described in this thesis were compared with data in the literature. They are discussed in the following overview with reference to future directives.

Dry fractionation

A first way to enhance nutritional properties of bean diets is to prevent the presence of ANF in feed. As one approach, the distribution of lectins and trypsin inhibitors was studied after air classification of bean flour. This technique fractionates legumes into their main constituents, protein bodies and starch granules.

The yield and composition of the different fractions depend on the processing conditions used for milling and air classification (Wright *et al.*, 1984; Sosulski *et al.*, 1987). The effect of the type of milling and the used cut point (limitation of the particle size at fractionation) during air classification was studied (Chapter II). The results showed that the protein levels in the fines fractions were increased to at least double that of the initial flour. This indicates that air classification of bean flour is suitable to obtain bean protein concentrates. The processing conditions under investigation, however, were not succesful in effecting a clear separation of the proteinaceous ANF from bean protein itself. This may indicate that the ANF are not distinguished from the bean protein itself with respect to physical characteristics. These ANF were accumulated in the fines (protein) fractions at levels similar or higher (when expressed on a protein basis) compared to the initial flour. Compared to the flour, the coarse fractions contain lectins at levels which may still be deleterious. The lectins from *Phaseolus* present in these fractions have been shown to evoke various morphological changes in cultured explants of small intestinal mucosa (Chapter II). This effect is no doubt due to the binding of *Phaseolus* lectin (King *et al.*, 1983; Kik *et al.*, 1989). The processing conditions used in Chapter II may be altered in such a way that the coarse fraction is almost free of lectins. However, it is the bean protein that is of concern. Air classification, thus, is not a feasible method to provide *Phaseolus* bean protein concentrates for feeding practices unless at least elimination of lectins can be obtained. Since lectins are proteins, they can be eliminated by thermal processing of beans. If thermal processing reduces the moisture level of beans, it will also increase the efficiency of air classification (Aguilera *et al.*, 1982; Reichert, 1982).

Thermal treatments

Introduction

The potential utilization of plant materials which have been subjected to thermal treatments requires a study on their chemical as well as nutritional consequences, particularly of proteins. The major objectives in the heat processing of dry beans were (1) to eliminate lectins and, simultaneously,

protease inhibitors and (2) to increase the digestibility of protein.
Proteinaceous ANF such as lectins and protease inhibitors require their structural integrity for their activity and therefore they will be inactivated by heat (Reaidi *et al.*, 1981; Rackis *et al.*, 1986). Since heat processing can be done with several instruments under various conditions, the effectiveness is sometimes questioned (Prince *et al.*, 1988, cited in Pusztai, 1989). The negative effect of lectins in *Phaseolus* beans on the utilization of nutrients, particularly protein, is of overriding importance to any other nutritional deficiency from beans. This is primarily caused by their binding to membrane receptors of epithelium cells of the small intestine and in addition by their effects on immunity (Sgarbieri *et al.*, 1982; Pusztai, 1989). The interaction with small intestinal epithelium has been reported to be a primary cause for intestinal disturbance (Kik *et al.*, 1989).
Also, the utilization of storage proteins of beans and other legume seeds are suggested to show resistance to proteolytic attack (Liener and Thompson, 1980; Restani *et al.*, 1983). Heat denaturation may improve their proteolysis.
Finally, under severe heat treatment of protein sources, the protein molecule may change profoundly with a possible consequent reduction in the amount or the availability of the essential amino acids (De Wet, 1982). In studies aiming at maximizing nutritional value of bean proteins, this has to be taken into account.

Thermal processing of *Phaseolus* beans can be done by various heating techniques. (Chapter III). The processing of beans is done conventionally by heating with steam in a cascade-type toaster at a more or less defined combination of temperature (~102°C) and time. Depending on origin, beans differ with respect to their antinutritional properties (Chapter I). Therefore, an adjusted processing time needs to be chosen for each product to result in a sufficient inactivation of lectins to insure, at least, a non-toxic final product. For quality control, the measure of lectin inactivation, based on haemagglutination (HA) with red blood cells, is used in practice.

It was noted (Chapter I) that the inactivation of ANF has been studied extensively for e.g. cooking procedures and autoclaving. These techniques do not simulate actual treatment procedures in animal feed technology with respect to the moisture level and practicability, respectively. In Chapter III these and other techniques have been discussed with relevance to decisive processing variables, the design and some consequences for practicability.
Variations of processing variables such as time, temperature, particle size and shear will alter the characteristics of the final product (Seerley *et al.*, 1974; Rackis *et al.*, 1986).

It was deduced that ANF inactivation can be established within short times when heating is performed in pressurized equipment.
A laboratory-scale pressurized toaster was built to provide controlled conditions for temperature and time for bean processing. This permits the use of short processing times at high temperatures.
The evaluation of steam heating procedures for beans by chemical, enzymic and biological assays was studied in the experiments described in the Chapters IV, V and VI.

Analytical estimates
So far, steam processing has not been systematically studied in literature. Most studies in literature aimed at studying the inactivation of lectin and trypsin inhibitor activity (TIA) at fixed temperatures in dependency of the processing time (Chapter I). In Chapter IV the effect of steam treatment on the protein quality and on ANF inactivation as obtained using the methods described in Chapter III have been evaluated. Moreover, the level of lectins was assessed by the determination of total and functional lectins using sensitive assays, which have recently become available (Hamer *et al.*, 1989). Commercially available beans were steam processed with different operating conditions without prior pre-processing. An analytical approach was used to calculate and to predict quality changes.
It was demonstrated that the rate of TIA loss from beans was temperature dependent. TIA inactivation, however, did not conform according to a single reaction following first-order kinetics. At each temperature which was investigated, a biphasic curve was found indicating two different reactions. This has also been observed for TIA inactivation in whole soybeans (Collins and Beaty, 1980; Liener and Thomlinson, 1981). Change in the nutritional value of beans after various conditions of steaming may differ with respect to lectin contents and TIA. It is uncertain whether the residual TIA totally reflects the trypsin inhibitors present in beans. Multiple forms of inhibitors might have been present as reported in *Phaseolus* beans (Tsukamoto *et al.*, 1983). Residual TIA reflects the total reduction of one or more inhibitors because with the used TIA assay, TIA does not discriminate between the activity from TI and from non-specific inhibition by e.g. tannins, fatty acids and phytate (Liener, 1989; Roozen and De Groot, 1989).

The levels of total lectins (TL, ELISA) and functional lectins (FL, FLIA; functional lectin immunoassay) were determined. For the FLIA, brush border membrane (BBM) or fetuin was used as adhering agent. The levels of TL and FL in beans were greatly reduced by steam treatments. It is noted that with

atmospheric steaming the inactivation of TL and FL (BBM) follows a similar pattern whereas FL (fetuin) inactivation is established at a slower rate. The rate of lectin inactivation was found highly temperature dependent. In practice, beans are generally heated for 30-40 min. at 102°C. That much steam processing left about 8 percent of the original TL level as a residual (Chapter IV, Table 3). At 119°C and 136°C the shortest processing times under investigation revealed a much lower level of residual TL.
The assays used for lectins are based on the binding of lectin tetramers. ELISA measures all tetramers, due to the cross-reactivity of *E*- and *L*-type anti-lectin antibodies. With FLIA, functional intact lectins are determined, where functionality is related to their binding to sugar (fetuin) or brush border membrane (BBM). In Chapter IV it was found that the ELISA assay may give a good indication for the total lectin content since it still can discriminate between very low levels. ELISA may be chosen also because it shows better specifity and sensitivity as reported by Hamer *et al.* (1989). Compared to the ELISA, assays for functional lectins (FLIA), at present, are less sensitive. Firstly, the coating of a sugar matrix (fetuin) is less effective than the coating of an antibody in ELISA; secondly, binding as an antibody-antigen complex is probably stronger than the binding in a lectin-carbohydrate complex (Van Oort, 1989; pers.comm.). Moreover, the sensitivity of the FLIA-fetuin is influenced by other factors such as incubation time and the type of sugar matrix. Further investigations on the relationship between the binding and pathological effects of lectins *in vivo* are needed to establish the actual contribution of the FLIA to serve as an indicator for effects of bean lectins.
It was found that the HA of the heated samples was more variable compared to the other assays in use for lectin analysis. The discrepancy of these results may be attributed to the sensitivity of the HA-assay since it is dictated by its reading precision which is at least one step in serial dilution. Therefore, HA may not be a good predictor for the level of total lectins.

Processing times, while ensuring more or less complete destruction of heat sensitive ANF may result in nutritional damage (Bender, 1984). It was shown that short processing at 119°C, needed to inactivate ~95% of lectins, resulted in a relatively small decrease in lysine availability determined with trinitrobenzenesulfonic acid (TNBS). This has been reported also from other observations on the level of available lysine in heated beans for FDNB (fluoro-dinitrobenzene) lysine availability (Molina *et al.*, 1976; Almas and Bender, 1980).
It was shown that for steam processing of dry beans the rate constants of ANF were higher compared to those of lysine parameters. ANF and lysine differ with respect to their temperature dependency. Therefore, processing at a high

temperature for a short time (HTST) is preferred in order to obtain high protein quality.
Heat penetration velocity will increase with a smaller particle size diameter of the beans. The latter is of particular concern to such heating conditions where only very short processing times are applied. It might therefore prove useful to study the inactivation by HTST-processing with beans, roller milled prior to heat processing.

In vivo estimates
A further evaluation of the effects of steam processing on bean protein quality arises from the relationship between the investigated processing conditions and the biological response in the animal. Species differences have been observed in the degree of negative effects when inadequately processed beans are fed. Therefore the steam processing effects were evaluated by digestibility measurements in pigs.

The overall protein digestibility of beans after heat treatment was estimated in *in vivo* experiments measuring apparent digestibility. "Apparent" digestibility has been referred as the net disappearance rate at the terminal ileum or at the end of the intestinal tract. References made in literature to "availability" or "apparent absorption" have actually the same meaning when determined from values for nutrient disappearance along the intestinal tract (Bielorai *et al.*, 1983). The apparent digestibility of protein or amino acids in pigs can be determined at faecal and ileal levels. At present, the ileal method is the preferred method for the determination of protein and amino acid digestiblity in feedstuffs for pigs (Tanksley and Knabe, 1984; Moughan and Smith, 1985; Knabe *et al.*, 1989). The ileal method is thought to be a better estimate since digestibility is measured prior to microbial degradation and synthesis of amino acids in the large intestine (Sauer and Ozimek, 1986). Moreover, ileal digestible crude protein and amino acids have been found to have higher correlations to deposited protein compared to their determination from faeces (Dierick *et al.*, 1988).

Using the ileal method, the digestibility of a feedstuff is determined by difference with a basal diet. The possible interactions of the feedstuff with this basal diet are assumed to be absent. Moreover, it is assumed that the disappeared amino acids are absorbed as amino acids being as such available in metabolism.
The determination of "true" digestibility implies a correction for endogenous nitrogen. When the traditional methods are used to calculate true digestibility, no interaction is assumed between endogenous protein and the level of dietary

protein or the composition of the diet. The use of "true" digestibility values by correcting the apparent digestibility values for losses of metabolic and endogenous nitrogen as estimated by e.g. the feeding of protein-free diets may mask differences between feedstuffs or processed feedstuffs (Muztar and Slinger, 1981; De Lange *et al.*, 1989). In this respect, the ^{15}N-isotope dilution technique (Souffrant *et al.*, 1986) has been proposed as a more reliable method for measuring endogenous secretion (De Lange *et al.*, 1989). However, this technique is not readily available since it requires ^{15}N labelled amino acids and specific measuring equipment. Future developments will undoubtedly increase the use of this technique for measuring true digestibility. Until then, the apparent ileal digestibility of proteins and amino acids remains a practical criterion for evaluation of protein quality for specific animals and diets.

It was shown that diet inclusion of beans, steamed at 102°C for 20 min diet significantly increased the amount of ileal chyme. It also decreased the digestibility of nitrogen and lysine at the end of the small intestine (Chapter V). During this initial stage of steaming lectins were reduced to ~20% and the TIA to ~15%. These processing conditions are likely to be insufficient to eliminate completely the negative effects in the intestinal tract. This was also previously shown for daily gain and feed conversion efficiency in young piglets (Van der Poel *et al.*, 1990). Processing at 102°C, for longer than 20 min increased ileal digestibility of nitrogen, which was, however, still lower than the value in the control diet in which casein and herring meal were included. We calculated that the nitrogen digestibility of beans, heated at 102°C for 40, 60 or 80 min was only ~30% at the ileal level. Obviously, at 102°C, prolonged heating does not contribute to a higher protein digestibility. The digestibility values of beans, following the classical (faecal) method, were calculated to range between 40 and 50%. This estimate is similar to the coefficient (0.44) observed for protein digestibility of steam heated white beans reported by Rodriguez and Bayley (1987). The results show that the improvement of dry matter digestibility by heating is more clear from data obtained by ileal than by faecal digestibility. For nitrogen digestibility, effects of heating on ileal and faecal are similar to a large extent.
A comparison was made of beans, heat processed at 102, 119 and 136°C during times which had previously shown to inactivate lectin (by ELISA) and TIA to a similar low level. HTST steaming procedures of beans resulted in higher digestibility of protein or lysine from beans. Based on the studies described in Chapters IV and V it was derived that the beneficial effect on the bean protein occurs simultaneously or in addition to ANF inactivation (Figure 1).

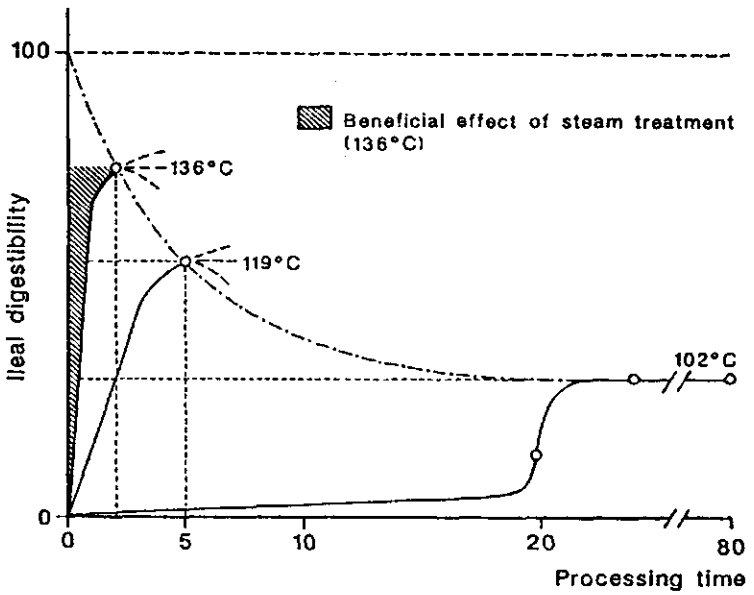

Figure 1. The effect of temperature and time with steam heating on the ileal digestibility of beans (*Phaseolus vulgaris* L.). (Solid line = temperature effect; o = measuring point; ▨ = maximum improvement)

Processing at 136°C for 1.5 min even did restore ileal N digestibility in bean diets (Chapter V, Table 5) to the level of the control group ($P<0.05$). These results lead to the conclusion that the low level of both lectins and TIA in beans, after steaming at these different conditions, cannot be the main reason for the relatively large differences in ileal digestibility found for these beans. These results indicate that, in the absence of ANF, other factors are involved in the different response due to variation in the temperature-time relationship in processing. Presumably, changes of the storage protein (Glycoprotein II) molecule with HTST-treatment contribute to higher digestibility values, an effect, that is not observed for (prolonged) steaming at 102°C. Wright and Boulter (1980) have indicated, that the denaturation temperature of Glycoprotein II is ~95°C for meals suspended in 1-2 times their weight in buffer or water. However, the temperature of the denaturation process increases with decreasing water content. For the major proteins of soya, the denaturation temperature was found to increase by over 30°C as the water content of the system decreased from 90 to 20% (Sheard *et al.*, 1986). This means that denaturation of the protein molecule probably has a beneficial

effect on digestibility. If the same would be the case for *Phaseolus* bean protein, the denaturation temperature would be about 125°C. This effect may differ between legume species as no beneficial effect was found for lupin protein in response to moist or dry heat (Batterham *et al.*, 1986). Although *in vitro* studies showed that heat denatured protein may be readily hydrolysed (Chapter I), these results have been questioned (Sgarbieri *et al.*, 1982). Moreover, *in vitro* digestibility of heat treated *Phaseolus* beans as measured by two enzymatical procedures showed no acceptable estimates for absolute values or ranking of nitrogen or dry matter digestibility (Chapter VI).
A further explanation for the improved digestibility of beans steamed at higher temperatures may be that prolonged heating at 102°C includes conditions, which negatively influence proteolysis. Also, the presence of an unidentified factor which would appear to be sensitive to heat only at elevated temperatures >100°C could be a responsible factor. Compared to globulin, only a few studies have dealt with the digestibility of the albumin fraction. Unlike globulin, albumin was observed to be less digestible (*in vitro*) following heat treatment, but the extent differed with the enzyme(s) used (Marquez and Lajolo, 1981).
The observed loss of protein dispersibility after heating (PDI, Chapter IV) suggests that some conformational and/or aggregational changes of protein may have taken place. The differences in PDI loss between the treated beans, however, are relatively small. In addition, PDI values have a tendency to rise again after prolonged heating at 136°C (Chapter IV). This phenomenon requires further elucidation. The additional occurrence of interactions between proteins and carbohydrate and the occurrence of the so-called browning reaction emphasize the elucidation of a multiplicity of phenomena that can occur at elevated temperatures or under 'adverse' thermal conditions.
It is believed that no single phenomenon can account for the different effects of thermal processing observed for *in vivo* digestibility of heated beans. The exact mechanisms as well as the magnitude at high temperatures, therefore, require further investigation.
As mentioned, the apparent ileal lysine digestibility varied with variation in steam treatment procedures for beans (Chapter V). In general, the same digestibility pattern for lysine and nitrogen was found. The TNBS assay values decreased with heating time (Chapter IV), indicating the binding of the free ϵ-aminogroup of lysine, suggesting that available lysine was reduced.

Based on the *in vivo* ileal digestibility results, the thermal treatments used (Chapter IV and V) cannot be termed 'overheating'. However, some caution is warranted because utilization of absorbed amino acids may still be affected negatively by thermal treatments e.g. due to intestinal absorption of products

of the early Maillard reaction as reported by Gall (1989) and Moughan *et al.* (1989) for animal proteins. If N excretion in urine is increased with HTST-protein uptake, this may be an indicator for reduced utilization of absorbed amino acids. However, this was not investigated. It cannot be excluded that with HTST-processing, extra N in urine would arise from absorbed but unavailable lysine. Other amino acids, however, may still be more available for the animal. It seems therefore worthwhile to measure the total of urinary N in future evaluation with respect to the heat processing of feedstuffs.

Prediction of in vivo digestibility

To obtain optimal processing variables the measurement of *in vivo* digestibility criteria is necessary. One of the best estimates, the measurement of ileal digestibility value, however, has some practical drawbacks. This technique is costly, time consuming and only a few treatments can be evaluated. Therefore, the use of methods which have high correlations with *in vivo* digestibility estimates and which can be easily done in a large number of samples, are useful.

The Mobile Nylon Bag Technique (MNBT; Sauer *et al.*, 1983) the advantages of which are described in Chapter VI, as well as two *in vitro* enzymatic methods were used to obtain data for the evaluation of technological treatments and/or to predict protein digestibility.

There was much less variation in the digestibility values obtained by the MNBT, pH-stat and IVPD procedures (Chapter VI, Table 5) in comparison with values obtained by total collection of ileal chyme. This is not uncommon for MNBT or *in vitro* enzymatic methods (Sauer *et al.*, 1989; Babinszky *et al.*, 1989). The latter methods will be useful if ranking of observed digestibility values would have a high correlation with *in vivo* values (Sauer *et al.*, 1989). It was shown that correlations for ileal *in vivo* (conventional method) and MNBT was highest for N ($r=0.96$; $P<0.01$) and dry matter ($r=0.88$; $P<0.05$). Nitrogen digestibility determined by the *in vitro* pH-stat/IVPD methods (Pedersen and Eggum, 1983; Babinszki *et al.*, 1989) showed no relationship with ileal N digestibility *in vivo*. *In vitro* enzymatic techniques use a surplus of enzymes and, in some procedures, a relatively long incubation time is applied. Therefore, these techniques may have lost sensitivity to certain physical and chemical characteristics of the material assayed (Pedersen and Eggum, 1983) and require further refinement particularly aimed to obtain assay conditions for higher discriminating ability. The MNBT is closer to the animal. It involves *in vitro* incubation and *in vivo* digestion from duodenum to terminal ileum and the enzymes and conditions of the intestinal tract are used. The MNBT showed to discriminate between samples treated at different temperatures and with different durations of steaming.

In general it was found that there were no linear relations between *in vivo* digestibility estimates and the level of lectins or TIA in beans present after heating. This again indicates that in addition to alterations caused during inactivation of these ANF digestibility is influenced by other mechanisms. It is notable, that the protein dispersibility index (PDI) as a chemical estimate has a good linear relation with *in vivo* N digestibility. A negative correlation ($r = -0.84$; $P<0.01$) can be calculated. This means that beans with a low PDI value showed a high digestibility. An increase of PDI values, however, found after prolonged heating at higher temperatures (Chapter IV), implies a careful interpretation of the use of the PDI as a parameter used for ranking and for quality control. It is concluded that the effects of processing at higher temperatures still need to be quantified.

General

Heat treatments of beans in the range of temperatures between 125 and 145°C compared to those of about 100-110°C increased weight and feed conversion efficiency when used in feeds containing whole soybeans either roasted (White *et al.*, 1989) or extruded (Seerley *et al.*, 1974). The mechanisms underlying these effects, however, have not yet been investigated.

Short time steam processing at 136°C was effective to inactivate virtually all the TIA and the lectins, as assayed by ELISA. With the use of these conditions for steam processing, the conformational changes of proteins are likely to be of greater importance than the concern of antinutritional factors.

This also implies, that the value of the ANF threshold levels in HTST-processed *Phaseolus* beans for the digestibility of bean protein is of limited value. Threshold values are normally used to give limits for maximum inclusion of feedstuffs into diets. In the case that the protein value of heated beans has no relationship with the residual levels of ANF, threshold values cannot be used to calculate the contribution of this diet ingredient to the protein value of the diet.

It has been often stated that HTST processing may be either on the verge of an 'insufficient treatment' and an 'excessive treatment'. This statement, however, needs to be reconsidered in the light of the methods used to determine the effect of processing. The results of the present studies clearly indicate that with different temperature-time combinations during steam treatment a sufficient inactivation of lectins is established. Moreover, large differences were found between ileal digestibility coefficients determined *in vivo*.

Qualitative aspects of bean protein processing have not been included in our experiments. A reduced lysine availability (TNBS assay) may result in the absorption of lysine, of which not all is available for metabolism. In that case, the impact of heat processing on available lysine *in vivo* needs to be established. The implications for 'excessive' treatments can then be discussed also. A better N utilization depends also on the amounts of amino acids which can be used for metabolism in addition to lysine. It has been found for instance that lysine is more heat sensitive than other amino acids. Therefore, it needs to be evaluated to what extent other amino acids are affected by heat treatments. If only lysine availability is reduced, simple supplementation of the diet with extra lysine would enable a better utilization of feed nitrogen from HTST-processed beans by the animal. In addition, the optimal processing conditions can be defined by criteria based on the effect which is considered most important.

In general a major shortcoming of studies aimed at the effect of thermal treatment on the inactivation of ANF, enzymes and, consequently, nutrient retention is that the experiments required to establish the kinetic models are time consuming. Much research, therefore, has been devoted to the development of an accelerated method which provides a rapid assessment of physical and chemical changes during heat treatments. Differential scanning calorimetry (DSC) is a thermo-analytical technique for monitoring these changes as a function of temperature. It can provide denaturation characteristics of a particular protein (Wright and Boulter, 1980; Biliaderis, 1983). The use of DSC, therefore, may be used to study the effects of thermal treatments on proteins.

Conclusions

The concerns of antinutritional factors present in beans (*Phaseolus vulgaris* L.) which can be used in animal nutrition are firstly the difficulty for labelling commercially available seeds to their antinutritional properties. The identification of ANF is laborious and often there is also contamination with other samples. Therefore, all beans have to be considered potentially toxic. Secondly, an increase of the *in vivo* protein nutritional value of beans after heating does not allow the estimation of the negative nutritional significance of a specific ANF. This is due to the presence in the same legume seeds of several different ANF.
In the present series of experiments quantitative aspects of lectins and trypsin inhibitor activity have been reviewed and investigated. It is concluded that

lectin proteins from bean may result in pathological changes of the small intestinal mucosa and therefore may interfere with digestion and absorption of nutrients. They may cause a considerable reduction in apparent digestibility of protein and lysine from beans as measured from determination of nutrient disappearance during passage through the digestive tract.

It was not possible to separate storage proteins from lectins and TIA in flours by means of air classification. Lectin proteins as well as TIA can be reduced rather rapidly by thermal treatment (steam processing) of dry beans. Conditions for inactivation of lectins and TIA have been investigated. Based on the kinetics for ANF inactivation and total lysine retention, it is suggested to employ HTST-procedures rather than LTLT-procedures for steaming of beans. The former conditions will increase the *in vivo* protein and lysine digestibility as determined with pig experiments.

Based on the sensitivity and functionality for lectin proteins, the ELISA assay is preferred above the assay for haemagglutination activity. *In vitro* enzymatic and related techniques (MNBT; pH-stat and IVPD) used as a rapid assessment do not predict the *in vivo* ileal digestibility values of HTST treated beans. The MNBT, however, gives a good correlation with *in vivo* estimates although the variation between treatments is less with MNBT. Although the MNBT offers promising possibilities, improving the existing *in vitro* techniques is of major importance.

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ABSTRACT

In animal production, feeding has an important impact on productivity and health of animals and feed composition is known to influence protein and energy metabolism directly. For monogastric animals complete diets are manufactured in which feed ingredients are used to supply the energy yielding and other nutrients. The common bean (*Phaseolus vulgaris* L.) is such an ingredient.
In common beans (*Phaseolus vulgaris* L.) the supply of nutrients is often lower than is expected from its chemical analysis and lower when compared to feed ingredients such as animal products. It has been shown in the present study that the utilization of nutrients from beans, particularly protein, is low due to the presence of so-called antinutritional factors (ANF). Moreover, there is a low digestibility of bean protein itself. Unless some form of processing is used, beans have little nutritional value for piglets. Various aspects of the processing of common beans and its evaluation were studied and the results reported in this thesis.

In the first chapter, a review of literature is given covering the available information on the reduction of ANF such as lectins and protease inhibitors in common beans by processing. In beans these ANF are present at either low or substantial levels. They have to be taken into account in the evaluation of bean protein quality. On the other hand, it is also the protein itself which is a component of primary interest.
It was found that the efficiency of the utilization of proteins for legume seeds depends on 1) the inherent protein resistance to proteolysis and 2) the interference by ANF, particularly the lectins.
The examination of literature revealed that research efforts so far have been directed particularly to define the processing variables for beans with respect to human consumption. This also implies the use of treatment procedures, which do not simulate the methods normally used in animal feed technology. Hence, the results of digestibility studies are difficult to compare.
It was decided to study the fractionation of beans and to investigate the distribution of ANF over the various fractions. In addition, the thermal processing of beans was studied systematically. Biological and chemical assays

were also used to estimate the effectiveness of processing.
The fractionation of dry beans was studied by means of particle size reduction by fine milling and subsequent air classification. This fractionates the seed in a fines and a coarse fraction in which protein and starch are accumulated, respectively. Different procedures for milling and classification yielded fines fractions with protein levels up to 52%, being at least twice the level of the initial flour (Chapter II). The protein level in the fines fraction varied, which was mainly caused by the classifier settings under investigation. Lectins and trypsin inhibitor activity accumulated to a large extent into the fines fraction to levels being one to four-fold those of the initial flour. Expressed on a protein basis this means that air classification leads to similar or higher levels of ANF in the fines fractions. The coarse fraction also contained considerable levels of ANF. Based on the pathological effects of the fines fraction as measured in cultured explants of small intestinal mucosa of pigs it was concluded that those fractions cannot be used in feeding practice without prior elimination of at least the lectins.
For the elimination of lectins and other proteinaceous ANF, various heating techniques can be used, each of which have their advantages or disadvantages. On the basis of literature, steam processing was chosen as a principle process for systematic research into the inactivation of lectins and trypsin inhibitor activity. Therefore, a modified steam processing equipment was developed to enable high temperature/short-term (HTST) processing in addition to low temperature/long-term (LTLT) processing. The advantage of pressurized steaming (HTST) was evident in that processing times could be shortened considerably (Chapter III). This was monitored by the reduction of enzyme activity in soya beans after steaming.
The effect of heat on the inactivation of antinutritional factors and on protein quality was evaluated by steam treatment of dry beans (Chapter IV). Different time/temperature combinations were used. The inactivation of the parameters investigated can be described by first-order reaction kinetics. However, inactivation of trypsin inhibitor activity (TIA) as well as the loss of protein solubility occurred in two stages with different reaction rates, with the initial stage having a higher rate of inactivation. Lectins from beans were rapidly inactivated at temperatures of 119 and 136°C as monitored by a sensitive ELISA assay (Chapter IV). A part of the total lysine was lost on heating but the amount of available lysine was reduced to a greater extent.
All parameters investigated were highly temperature dependent. The results of this study indicate the steam processing of dry beans at 119°C for 5 to 10 min. being good enough for ANF inactivation and not too excessive for protein damage as measured by total and available lysine.

The effects of steam treatment on bean protein quality were estimated *in vivo* in experiments in which apparent ileal digestibility was measured in piglets fitted with a post-valvular T-caecum cannula (PVTC) (Chapter V).
In each of two experiments a control diet was used that contained casein and herring meal as protein sources. In Experiment 1 the experimental treatments consisted of beans, steam heated under atmospheric conditions (102°C) for 20, 40, 60 and 80 min., respectively. Experiment 2 comprised six experimental treatments: beans steam heated at 102°C for 60 min.; at 119°C for 5 min.; at 136°C for 1.5 min. and inclusion at 10 and 20% into the piglet diets, respectively. Intact animals were used to determine faecal digestibility of nitrogen in the same experimental treatments.
In Experiment 1, ileal digestibility of lysine and nitrogen and faecal digestibility of nitrogen were reduced significantly by the inclusion of beans, steam heated at 100°C for 20 min. Digestibility of nitrogen and lysine increased in bean containing diets with longer processing times of beans at 102°C. In all cases, digestibiity coefficients of bean diets remained below the control diet.
In Experiment 2, the inclusion of 20% beans, processed at 119°C or 136°C resulted in an increased ileal digestibility of nitrogen compared to atmospheric steaming for 60 min. Steam processing, at 136°C for only 1.5 min. on the other hand, did restore ileal nitrogen digestibility to the level of the control group (Chapter V).
These studies indicate a marked difference in the biological response of piglets to diets containing beans exposed to prolonged steaming at 102°C and to HTST-processing of beans. Based on the ileal apparent nitrogen digestibility, it was concluded that HTST processing was more favourable than LTLT. The treatments of beans used in experiment 2 inactivated lectin and TIA both to a similar low level. HTST processing also resulted in a higher protein digestibility. This indicates a more pronounced role of the properties of the bean storage protein in explaining a much higher nitrogen digestibility *in vivo* after HTST-processing. This phenomenon requires further investigations.
Technological treatments generally involve a large number of processing variables. This also gives a large number of samples to be tested. It is important therefore to assess the potential value of alternative digestibility techniques which are more rapid to perform and which are less costly. Studies were made to evaluate the mobile nylon bag technique (MNBT) and two *in vitro* methods for determining and to predict the apparent ileal dry matter (DM) and nitrogen (N) digestibility (Chapter VI). A variety of steam heated bean samples was used and a comparison was made with *in vivo* digestibility values obtained previously by the conventional digestibility method involving total collection of ileal chyme (Chapter V).

The *in vitro* enzymatic procedures showed that from the procedures tested no acceptable estimates for absolute values of N and DM digestibility were obtained. Significant correlations, however, were found between MNBT ileal digestibility of N and DM and the *in vivo* ileal digestibility coefficients. The MNBT technique has certain advantages such as the requirement for only small amounts of feed samples and the determination of digestibility of different samples within the same animal. In this context, the MNBT appears to be promising for a rapid prediction of ileal digestibility of thermally processed feedstuffs. A disadvantage of the technique is that it requires surgically modified animals. Modification of the existing *in vitro* techniques, therefore, is necessary, so that their prediction value for digestibility in ANF containing feedstuffs improves.

SAMENVATTING

In de voorziening van veevoeders ten behoeve van de intensieve veehouderij wordt in Nederland vrijwel uitsluitend gebruik gemaakt van industriëel bereide mengvoeders. Tesamen met water kan door middel van deze zogenaamde 'volledige voeders' geheel in de behoefte van de varkens en het pluimvee worden voorzien. Deze volledige voeders worden dan ook samengesteld volgens bepaalde nutritionele en technologische normen teneinde het dier optimaal te voorzien van met name eiwitten en energie.

Mengvoeders worden samengesteld uit een groot scala van grondstoffen. Grondstoffen bevatten de noodzakelijke nutriënten, echter, ze kunnen ook toxische bestanddelen bevatten, waardoor deze grondstoffen niet of in slechts beperkte mate kunnen worden benut. Met name peulvruchten en oliezaden bevatten bestanddelen, waaraan een negatieve invloed wordt toegeschreven, indien zij in onbewerkte vorm zijn verwerkt in mengvoeder.
De teelt van peulvruchten in de EEG wordt op dit moment gestimuleerd zodat het gebruik van peulvruchten als grondstof voor mengvoeders economisch aantrekkelijk is.
Erwten, veldbonen, soyabonen, linzen, lupinen en voerbonen (stamslabonen) zijn peulvruchten die, geteeld in Europa, tot goede opbrengsten kunnen leiden. De groep van voerbonen (*Phaseolus vulgaris* L.) kent zeer veel variëteiten. Deze bonen verschillen aanzienlijk in grootte, vorm, kleur en chemische samenstelling. Voerbonen zijn met name belangrijk door hun relatief hoog eiwitgehalte en het gehalte aan zetmeel. De voederwaarde van deze bonen wordt echter beperkt door de aanwezigheid van toxische en andere zogenaamde antinutritionele factoren (ANF). Deze ANF kunnen de eiwitbenutting van voerbonen beïnvloeden. Deze beïnvloeding wordt geassocieerd met lectinen en enzymremmers alsmede, in enkele variëteiten, met tanninen. Lectinen zijn eiwitten die de eigenschap hebben zich te binden aan glyco-conjugaten, die gelocaliseerd zijn in de apicale celmembraan van dunne-darm epitheel van bv. jonge varkens. De lectinen zijn gedeeltelijk resistent tegen enzymatische afbraak in de darm. De binding van deze lectinen aan de darmmucosa kan verscheidene morphologische en functionele veranderingen in het darmepitheel tot gevolg hebben. Deze veranderingen

hebben consequenties voor de vertering en absorptie van nutriënten en kunnen uiteindelijk, afhankelijk van het soort lectine, zelfs lethaal zijn voor het dier. Enzymremmers, met name remmers van het enzym trypsine, veroorzaken een onvolledige afbraak van voedereiwitten in het darmkanaal.
De verteerbaarheid van het boneneiwit uit de *Phaseolus vulgaris* L. soorten is relatief laag, hetgeen geassocieerd wordt met de antinutritionele eigenschappen van de eiwitten.
De meest gangbare methode om voerbonen voor verwerking van mengvoeders geschikt te maken is de toepassing van een warmtebehandeling. Als gevolg van hitte wordt een aantal ongewenste stoffen (eiwit-ANF) gedenatureerd en daardoor inactief gemaakt. Ook het eigenlijke boneneiwit ondergaat zelf veranderingen; echter de consequenties daarvan voor de vertering staan niet geheel vast.
De effectiviteit van een warmtebehandeling m.b.t. de voederwaarde van peulvruchten hangt af van een combinatie van proces-factoren zoals procestemperatuur, procestijd, de deeltjesgrootte van het te behandelen produkt en het vochtgehalte. Het beheersen van deze factoren is nodig voor het bereiken van die procesomstandigheden die optimaal zijn voor bv. het inactiveren van ongewenste stoffen. Een warmtebehandeling kan echter ook nadelige effecten hebben op sommige bestanddelen, zoals bv. op essentiële aminozuren met name lysine. Er moet zodoende een oplossing gevonden worden voor procescondities die enerzijds gewenste bestanddelen intact laten en anderzijds de ongewenste bestanddelen voldoende inactiveren.
De gehalten van lectinen en andere ANF worden veelal m.b.v. (bio)chemische methoden gemeten. Deze methoden kunnen evenwel de evaluatie van produkten zoals gemeten in dierproeven, nog niet volledig vervangen. Het is daarom zinvol om de verkregen resultaten van een (bio-)chemische evaluatie van technologische processen te relateren aan de resultaten verkregen uit dierproeven.
De doelstelling van het onderzoek beschreven in dit proefschrift is het effect te onderzoeken van technologische behandelingen van voerbonen op o.a. de mate van inactivering van enkele antinutritionele factoren en de eiwitverteerbaarheid in het dier.

In een literatuuroverzicht zijn de mogelijkheden beschreven om antinutritionele factoren in voerbonen te reduceren. De antinutritionele factoren komen in deze bonen voor in kleine (enzymremmers) tot aanzienlijke (lectinen) hoeveelheden. Het literatuuronderzoek toonde aan, dat de doelmatigheid van het benutten van eiwit afkomstig van bonen afhangt van (1) de mate waarin het boneneiwit aantastbaar is voor proteolytische enzymen en (2) de interactie van m.n. lectinen en de mucosa van de dunne darm. Uiteraard

speelt ook de eiwitsamenstelling in termen van aminozuren een rol.
Het onderzoek naar de inactivering van ANF in voerbonen zoals in de literatuur is beschreven, heeft zich met name toegespitst op het vastleggen van procescondities in relatie met de humane consumptie van bonen. Dit betekent, dat vooral processen zijn bestudeerd, die niet of nauwelijks overeenstemmen met de methoden die geschikt worden geacht voor toepassing in de veevoederindustrie. Als gevolg hiervan zijn de resultaten van deze studies niet altijd te gebruiken.
In het experimenteel onderzoek, beschreven in dit proefschrift, is allereerst de verdeling van lectinen en trypsine remmers bestudeerd na het fractioneren van bonen. Tevens is de thermische behandeling van bonen systematisch onderzocht en de effecten ervan beoordeeld aan de hand van chemische methoden, *in vitro* onderzoek en onderzoek in dierproeven.
Het fractioneren van droge bonen werd onderzocht door verkleining van de bonen tot zeer kleine deeltjes en deze aansluitend te scheiden met behulp van een windzifter. Deze laatste behandeling scheidt de boondeeltjes in een fijne en meer grove fractie. In de fijne fractie accumuleert het grootste deel van het eiwit en het eiwitgehalte loopt op tot ca. 52%. Dit gehalte is meer dan twee maal zo hoog als het eiwitgehalte in de oorspronkelijke boon en bleek met name afhankelijk van de gebruikte instelling bij het ziften. De lectinen en trypsine remmers (gemeten als trypsine remmende activiteit) accumuleerden eveneens voor een zeer groot deel in de fijne fracties, tesamen met het eiwit. Uitgedrukt per eenheid eiwit werd gevonden, dat de gebruikte technieken leiden tot gelijke dan wel hogere gehalten aan ANF in de fijne fracties.
In de resulterende grove fracties accumuleert het grootste deel van het zetmeel. Echter ook werden in deze fracties nog aanzienlijke ANF gehalten gevonden. De pathologische effecten van de fijne fractie werden onderzocht in gekweekte monsters van de dunne-darmmucosa van het varken. Geconcludeerd werd dat deze fracties als zodanig niet voor vervoedering in aanmerking komen tenzij in ieder geval de lectinen uit bonen worden geëlimineerd.

Verschillende technieken voor warmtebehandeling zijn in principe toepasbaar. Het verhitten met stoom (toasten) werd gebruikt als principiële warmtebehandeling in het systematisch onderzoek naar het effect van temperatuur en tijd op de inactivering van lectinen en enzymremmers. Ten behoeve van dit onderzoek werd apparatuur (op kleine schaal) ontwikkeld teneinde het proces te kunnen bestuderen bij hoge temperatuur gedurende korte tijd (HTST) alsook bij lagere temperatuur gedurende langere procestijden (LTLT). Het voordeel van de HTST behandeling (druktoasten) werd aangetoond in een experiment met het toasten van soyabonen. De

procestijd bij het druktoasten bleek aanzienlijk te kunnen worden teruggebracht door het toepassen van hoge temperaturen. Dit werd aangetoond aan de hand van de reduktie van de activiteit van het enzym urease bij verschillende temperatuur-tijd combinaties.

Het effect van een warmtebehandeling op de reductie van lectinen en trypsine remmende activiteit (TIA) werd onderzocht door droge bonen als zodanig te toasten. Als procesvariabele werd de temperatuur gevarieerd en bij elke temperatuur werden verschillende procestijden onderzocht. Alle onderzochte parameters bleken te kunnen worden beschreven volgens "eerste orde" reakties. De inactivering van TIA alsook de reductie in eiwitoplosbaarheid bleek echter door minstens twee achtereenvolgende eerste reakties te verlopen. Voor beide parameters geldt dat de reaktiesnelheid in de eerste fase aanmerkelijk groter was. Lectinen uit bonen werden zeer snel geïnactiveerd bij temperaturen boven de 100°C. Voor het bepalen van het lectinengehalte werd gebruik gemaakt van een recent ontwikkelde en gevoelige ELISA-methodiek. Als gevolg van de stoombehandelingen werd het gehalte aan lysine in beperkte mate verlaagd; het gehalte aan beschikbaar lysine, chemisch bepaald met behulp van trinitrobenzeensulfonzuur (TNBS), daalde evenwel sterker. Uit de reactiekinetiek zoals beschreven voor de verschillende onderzoekskenmerken blijkt, dat een stoombehandeling bij hogere temperaturen het meest geschikt is om de doelstelling te bereiken. Het toasten van droge bonen op een temperatuur van ca. 120°C gedurende 5 tot 10 minuten blijkt een goede middenweg in termen van een adequate inactivering van lectinen en TIA en van een minimale eiwitbeschadiging gerelateerd aan het gehalte van lysine en beschikbaar lysine.

Het effect van toasten op de eiwitkwaliteit van bonen werd vervolgens onderzocht in een tweetal dierproeven. Voor biggen werd de schijnbare ileale (droge stof, stikstof, lysine) en faecale (droge stof, stikstof) verteerbaarheid gemeten van getoaste bonen. In elk van de twee proeven werd een controle rantsoen gebruikt met caseine en haringmeel als bronnen van eiwit. In experiment 1 werd de verteerbaarheid gemeten van bonen, getoast bij 102°C gedurende resp. 20, 40, 60 en 80 minuten.
In dit experiment werden de ileale verteerbaarheid van stikstof en lysine alsook de faecale verteerbaarheid gemeten. De gevonden verteerbaarheden bleken significant verlaagd als gevolg van opnemen in het rantsoen van bonen, getoast bij 102°C gedurende 20 minuten. Een langere procestijd bij het toasten (102°) gaf een verbetering te zien van de ileale en faecale verteerbaarheid.
De absolute verteringscoëfficiënten van de bonen, getoast bij 102°C waren allen lager als die, welke gemeten werden voor het controle boonvrije rantsoen.

In experiment 2 werd een drietal behandelingen onderzocht. De bonen werden getoast bij 102°C gedurende 60 min., bij 119°C gedurende 5 minuten en bij 136°C gedurende 1.5 min.; deze bonen werden vervolgens in het proefvoeder verwerkt in percentages van 10 en 20 procent.
Uit deze resultaten bleek dat de verwerking in het rantsoen van bonen, getoast op hogere temperaturen (119°C; 136°C) een hogere ileale verteerbaarheid van stikstof en lysine in deze bonen tot gevolg heeft. De ileale verteerbaarheid van bonen, getoast bij 136°C bleek niet af te wijken van de waarden van het controle boonvrije rantsoen.
Met uitzondering van de stikstof verteerbaarheid van rantsoenen met bonen getoast bij 102°C werden geen significante verschillen gevonden tussen de ileale en faecale verteringscoëfficiënten van stikstof of lysine bij verwerking van 10 of 20 procent in het proefrantsoen. Uit de resultaten van deze proeven werd geconcludeerd dat er een groot verschil bestaat m.b.t. de verteerbaarheid van eiwit en lysine van bonen die getoast werden onder verschillende omstandigheden. Gebaseerd op de ileale verteerbaarheid voor biggen, blijkt een HTST behandeling voor bonen gunstiger te zijn. De warmtebehandelingen voor de bonen, die onderzocht werden in experiment 2, inactiveerden de lectinen en TIA tot vergelijkbare en zeer lage gehalten in de getoaste produkten. Dit betekent, dat bij opname van aldus behandelde bonen in het rantsoen door deze ANF geen significante effecten *in vivo* te verwachten zijn. De gevonden verschillen in verteerbaarheid duiden zodoende op de rol van andere factoren in deze, zoals bijvoorbeeld de eigenschappen van het boneneiwit zelf en het effect van warmtebehandelingen hierop. Nader onderzoek hiernaar is gewenst.

Technologische behandelingen leveren door het grote aantal procesvariabelen doorgaans een groot aantal behandelingsmogelijkheden op ten behoeve van onderzoek. Het is daarom belangrijk alternatieven voor verteringstudies te ontwikkelen en te onderzoeken op hun geschiktheid om effectief, snel en met lagere kosten de effecten van bewerkingen te kunnen evalueren. In het kader hiervan is de 'nylon zakjes' (MNBT) methode onderzocht als techniek voor het meten of voorspellen van verteerbaarheid. Tevens is de toepasbaarheid van twee *in vitro* enzymatische methoden onderzocht. In dit onderzoek zijn bonenmonsters gebruikt, die reeds onderzocht waren op hun *in vivo* verteerbaarheid. De gebruikte *in vitro* enzymatische methoden bleken geen acceptabele voorspelling te geven van de absolute verteringscoefficiënten noch van de rangorde ervan in samenhang met de stoombehandeling. Voor de 'nylon zakjes' methode echter, werd evenwel een goede correlatie gevonden tussen de 'nylon zakjes' verteerbaarheid van stikstof en droge stof en de *in vivo* verteerbaarheid zoals eerder vastgesteld in conventionele dierproeven.

Geconcludeerd werd dat de 'nylon zakjes' methode voor het vaststellen van verteerbaarheidscoefficiënten enkele belangrijke voordelen kent boven de conventionele methode. Er is slechts een kleine hoeveelheid van het te onderzoeken voermonster nodig terwijl b.v. de smaak ervan geen rol speelt. Tevens kan de verteerbaarheid van verschillende monsters binnen eenzelfde dier worden vastgesteld. Deze voordelen in aanmerking genomen is de 'nylon zakjes' methode een veelbelovende benadering voor een snelle voorspelling van de ileale verteerbaarheid van warmtebehandelde veevoedergrondstoffen.
Een nadeel van deze methode is het gebruik van gecannuleerde dieren. Verbetering van de huidige *in vitro* technieken is daardoor noodzakelijk, zodat de voorspelling van verteerbaarheid van grondstoffen met ANF, die met deze methode verkregen wordt, verbetert.

Curriculum vitae

Antonius Franciscus Bernardus van der Poel werd op 19 augustus 1953 geboren te Geldrop. Hij behaalde in 1971 het HBS-diploma en begon in datzelfde jaar met zijn studie Zoötechniek aan de Landbouwhogeschool in Wageningen.
Na het afstuderen in januari 1979 was hij werkzaam bij Cyanamid Benelux N.V. te Louvain-la-Neuve, België.
In februari 1984 keerde hij terug naar de huidige Landbouwuniversiteit bij de Vakgroep Veevoeding, waar hij als universitair docent dit proefschrift heeft geschreven.