IJAS Iranian Journal of Applied Animal Science

Effect of Extrusion and Conventional Processing Methods on the Levels of Anti-Nutrients Factors and Digestibility of Bitter Vetch (*Vicia ervilia*) Seeds in Broilers

Research Article

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Received on: 25 Dec 2013
Revised on: 16 Jan 2014
Accepted on: 30 Jan 2014
Online Published on: Dec 2014

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Online version is available on: www.ijas.ir

ABSTRACT

Comparative effects of extrusion cooking and conventional processing methods (cooking and autoclaving) on anti-nutritional factors and subsequent effects on *in vitro* and *in vivo* digestibility of *Vicia ervilia* in broilers were investigated. Treatments had significant effect (P<0.05) on chemical compositions so that decreased moisture, starch, crude protein, ether extract and crude fiber contents. Treatments of seeds resulted in significant reduction of total phenols, tannins, condensed tannins, canavanine and trypsin inhibitor activity (P<0.05). Treatments improved (P<0.05) *in vivo* digestibilities of dry matter, crude protein, true protein, starch and gross energy. Extrusion was the most effective method to reduction of anti-nutritional factors without modifying protein content. Furthermore, this thermal treatment was the most effective in improving protein and starch digestibilities when compared with soaking, cooking and autoclaving.

KEY WORDS anti-nutritional factors, broilers, digestibility, extrusion, vicia ervilia.

INTRODUCTION

Bitter vetch (*Vicia ervilia*) is known for its high nutritional value, capacity of nitrogen fixation, and ability to grow in poor soils (Farran *et al*. 2001). Bitter vetch seeds have been used in animal feeds and, when treated, as an alternative source of protein and starch in poultry diet (Farran *et al*. 2001). Raw bitter vetch, however, is detrimental to monogastric animals, especially chickens. The adverse effects arise from the presence of some anti-nutritional factors in the raw seeds (Aletor *et al*. 1994). Several detoxification methods have been evaluated for leguminous seeds, including acetic acid, sodium bicarbonate solutions and potassium bicarbonate solution, urea treatment and alkaline extraction. Additional techniques are the application of extrusion cooking which has advantages including high productivity, low operating costs, energy efficiency and shorter cooking times than conventional processing methods. Extrusion cooking application to legume processing has developed quickly during the last decade, and can now be considered as a technology of its own right. Legume extrusion cooking would allow reduction of anti-nutritional factors and therefore improve the nutritional quality at a cost lower than other heating systems (cooking, autoclaving, etc.) due to a more efficient use of energy and better process control with greater production capacities (Alonso *et al*. 2000). Therefore, in this study, it was aimed to evaluate effects of extrusion on anti-nutritional factors and subsequent effects on *in*

vitro and *in vivo* digestibility of bitter vetch seeds in broilers and comparison of results achieved with traditional processing (cooking and autoclaving).

MATERIALS AND METHODS

Experiments were performed at the farm of Agricultural, Medical and Industrial Research School, Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran (Karaj, Iran). All bird protocols were approved by the relevant Ethical Review Committee and all experimental conditions followed official guidelines for the care and management of birds. The seeds of bitter vetch were obtained from the agricultural and natural resources research institute, Sari, Mazandaran, Iran. Soon after its collection and removal of immature and damaged seeds, the mature seeds were dried in direct sunlight for 2 days and stored in plastic containers at room temperature (25 ˚C) until further use.

Processing methods

Sample preparation before cooking and autoclaving

Bitter vetch seeds free from immature and damaged seeds, dust and other foreign materials were soaked in distilled water (1:5, w/v) at room temperature (22 ˚C) for 12 h. The soaked seeds were drained and dried at 55 ˚C for 24 h. The dried samples were milled into flour (60-mesh size) and stored in air tight dark brown polyethylene bottles at room temperature (22 ˚C) until further analyses.

Cooking and autoclaving

After hydration for 12 h, the seeds were cooked in distilled water (100 ˚C) in a seed:water ratio of 1:10 (w/v) for 35 min. The cooked seeds were rinsed with distilled water and dried at 55 ˚C for 24 h in a hot air oven. For autoclaving, the clean seed samples soaked overnight in plain water were autoclaved (Model KT- 30LD, ALP Co., Ltd., Tokyo, Japan) with a temperature of 121 ˚C in plain water (1:3 w/v or 1 g:3 mL) for 30 min according to the method of Vijayakumari *et al*. (2007). Subsequently, the seeds were rinsed with distilled water, dried at 55 ˚C for 24 h in a hot air oven and milled in a cyclone mill of 60-mesh size.

Sample preparation before extrusion

Bitter vetch seeds soaked at the same conditions mentioned above. Then, seeds were ground without dehulling to a desirable range of particle size (200-500 mm) for extrusion. Before extrusion, the moisture content of whole seeds meals was adjusted to 22% by adding the required calculated amount of distilled water. The quantity of water was added slowly during stirring with a laboratory mechanical stirrer and then tempered by leaving in polyethylene bags at room temperature (22 ˚C) overnight to allow the moisture to equilibrate before extrusion.

Extrusion process

A Brabender Laboratory Single-Screw extruder equipped with feeding device AEV 300, speed control of the feeding device, temperature regulators for two extruder zones and die barrel head was used to prepare the extrudates. The barrel was divided into independent electrically heated zones (feed and cooking zones) cooled by air. A third zone, at the die barrel, was also electrically heated but cooled by water. The extrusion conditions were: temperature at cooking and die zones was adjusted together at 140 ˚C; screw speed 250 rpm; screw compression 4: 1; feeding screw speed 160 rpm and round die hole 3 mm. The resulting extrudates were allowed to reach room temperature, then sealed in plastic bags and stored at room temperature until analysis.

Starch isolation

The seeds were washed thoroughly, peeled and sliced into 2 mm thick slices using a rotary slicer and the slices were kept immersed in water containing 0.5% potassium metabisulphite to avoid browning. Defective slices were removed. The slices were ground thoroughly in a laboratory scale grinder to get fine slurry. The slurry was filtered through a muslin cloth and the residue on the muslin cloth washed repeatedly to recover starch. The filtrate was collected in a glass jar and left overnight for the starch to settle down. The supernatant liquid was decanted and the starch layer was washed repeatedly (4-5 times) with distilled water until the supernatant became clear. The starch cake was dried in a hot-air oven at 40 ˚C until dry. The dried starch was ground to a fine powder and kept in an airtight container at room temperature.

Chick bioassay

A total of 96 male, Ross strain broilers were selected. They were housed in pairs, within 10 g in weight (at 13-days) of each other. Broilers were allotted to cages in groups of 6. Prior to the adaptation and trial period, chicks were fed chicks starter crumb (AME: 11.7 MJ/kg; the following in g/kg; crude protein (CP): 190; ether extracts (EE): 33; Fibre: 33 g; Ash: 51; Ca: 9; available P: 4.5 and Lysine: 10). The experiment was carried out with 3 day adaptation period (days 19 to 21), 2 days starvation for depleting digestive tract, then 1 day feeding and following 2 days starvation for complete excretion of undigested material. The samples of dropping avoided during final 72 h period were collected, weighted and frozen (-18 ˚C). At day 19 the birds began an adaptation period, where they were fed the assigned trial diet (Table 1). The trial period then took place between days 23 and 27, a total of 96 h. During this time,

feed intake was measured and excreta collected. At all times, feed and water were provided on an ad libitum basis.

Chemical composition of the diets

Moisture content was determined from the samples before and after they were stored overnight in an oven at 105 ˚C (AOAC, 1995). Nitrogen was determined by using a Dosimat-776 Metrohm apparatus (Metrohm Co., Switzerland) according to AOAC (AOAC, 1995). The instrument was calibrated each time with ammonium sulphate as a nitrogen standard. Starch contents were determined on a spectrophotometer at 510 nm after extraction with boiling water, as described by some studies. Fat content was determined with a Solvent Extractor (Behr Labour-Technik, Düsseldorf, Germany) equipped with six Soxhlet posts (AOAC, 1995). Ash was determined by burning duplicate 2 g samples at 540 ˚C, for 3 h in a muffle furnace (AOAC, 1995). Crude fibre was determined by treating an oil-free sample by sulphuric acid (0.26 N) and potassium hydroxide (0.23 N) solution using an automatic fibre analyzer (Velp Scientifica, Milan, Italy), followed by oven drying and muffle furnace incineration (AOAC, 1995). Gross energy of seed and excreta samples were determined by adiabatic bomb calorimeter using Parr-4 Model 1241 Calorimeter. The True protein of the samples was quantitatively estimated following the method of Bradford (1976). The protein contents of the samples were calculated using a calibration curve obtained for bovine serum albumin standards (0-1.5 mg) treated in the same way. Two extractions were carried out per subsample and each sample was analyzed in duplicate.

Assay of anti-nutritional factors

Phenolics and tannins

Total phenolics of the seeds were assayed by adapting the method outlined by Rosset *et al.* (1982). A known amount of the seed flour (1 g) was extracted twice with methanol (50%, 5 mL) in a water bath (95 ˚C, 10 min). The pooled extract was made up to 10 mL, the extract (0.5 mL) was mixed with an equal quantity of distilled water and treated with 5 mL Na_2CO_3 (in 0.1 N NaOH). After 10 min, 0.5 mL Folin-Ciocalteu's reagent (diluted 1:1 with distilled water) was added and the color developed was read at 725 nm. The phenolics determined were expressed as gallic acid equivalents (GAE). The vanillin-HCl method was adapted to determine tannins in the seed flours (Porter *et al.* 1986). A known amount of the seed flour (1 g) was extracted with methanol (10 mL, 28 ˚C, 12 h), vortexed and decanted. This process was repeated and the supernatant was pooled and made up to 25 mL. The extract (1 mL) was treated with reagent mixture (5 mL) (4% vanillin in methanol and 8% concentrated HCl in methanol, 1:1). After 20 min, the colour developed was read at 500 nm (Spectronic 21, Miltonroy, USA) using catechin (50-250 µg) as standard. Condensed tannins were determined by butanol-HCl-Fe^{+} reagent (Porter *et al.* 1986). Condensed tannins were expressed as leucocyanidin equivalents.

Determination of canavanine

Preparation of pentacyanoammonioferrate (PCAF) reagent

Sodium pentacyanoammonioferrate (PCAF) was prepared by a procedure described by Cacho *et al.* (1989) as follows. 10 grams of sodium nitroprusside were dissolved in 55 mL of concentrated ammonia solution (32%). The solution was kept in the dark at 0 ˚C for 24 h. A yellow-green precipitate, containing a mixture of sodium pentacyanoammonioferrate (II) and (III), was filtered off and the filtrate was treated with absolute ethanol until complete precipitation had occurred. This precipitate was combined with the first precipitate and washed with absolute ethanol until all the ammonia had been removed. After partial removal of the ethanol by filtration, the precipitate was dried over H_2SO_4 and stored in the dark over $CaCl_2$ contained in a desiccator. It must be used within 48 h of preparation since, after this time, the PCAF begins to decompose, turning from its characteristic yellow colour to brownish green. Preparation of *Vicia ervilia* samples*:* two grams of a finely ground sample of bitter vetch seeds, which were defatted in a Soxhlet apparatus with petroleum ether, were extracted with 0.1 *M* HCl in the proportion of 1: 25 (w/v). The mixture was stirred on a magnetic stirrer for 6 h at room temperature and left overnight. The solution was centrifuged at 10000 × g for 20 min and supernatant was saved and the residue subjected to a second extraction for 6 h under the same conditions as the first. The combined extracts were adjusted to exactly pH 7.0 with 0.1 *M* NaOH solution and diluted to a final volume of 100 mL.

Determination of canavanine: One milliliter of standard canavanine (C-1625, Sigma Chemical Co., MO, USA) solution (1 mg mL^{-1}) was diluted with 0.1 *M* HCl to give concentrations which ranged from 0.005 to 0.08 mg ml^{-1} of canavanine.

In a 10 mL volumetric flask, to 1 mL of these diluted canavanine solutions, were added 6.5 mL of 0.2 M phosphate buffer (pH 7.0), 1 mL of 1% potassium persulphate and 0.5 mL of 1% aqueous PCAF (kept in dark) and the mixture was diluted to 10 mL with distilled water. The mixture was vortexed and, after 15 min, the absorbance was measured at 520 nm.

Similarly, an appropriate volume of sample solution, instead of standard canavanine, was used for the quantitative estimation. From the standard curve, the concentration of canavanine in the seed samples was determined and expressed on a dry matter basis.

Trypsin inhibitor analysis

Trypsin inhibitor activity (TIA) was determined according to Smith *et al*. (1980). Defatted ground seed samples (0.25 g each) were extracted for 5 min (2×2.5 min, with intermittent cooling in between the extractions, by keeping the tubes containing the samples in an ice bath) in 12.5 mL of 0.01 *M* NaOH at pH 9.4-9.6 using an Ultra-Turrax macerator (20000 rpm min^{-1}). The contents were centrifuged at 3800 × g for 15 min and the supernatants were collected. The supernatant was further centrifuged at 1000 × g, following which the supernatants were collected by slowly pipetting between the residue at the bottom and the fatty layer on top. These solutions were used for the assay after appropriate dilution with water.

***In vitro* digestibility**

Enzymatic digestibility by α-amylase was investigated for untreated and treated bitter vetch using the method described by Zhang *et al*. (1995) with some modifications. Starch (1 g, dry basis) was mixed with $KHPO_4$ / K_2PO_4 buffer (40 mL, 0.2 *M*, pH 6.9) in a test tube. The mixture was heated in a temperature regulated water bath at 90 ˚C for 40 min. It was cooled to 25 ˚C and 320 units of bacterial α-amylase *Bacillus licheniformis* (2 units/mg, Fluka) were added. Five replicate preparations were made for each sample, in order to monitor enzymatic digestibility with time. The tubes were placed in water bath and they were incubated at 30 ˚C between 10 and 26 h. H_2SO_4 (1.0%, w/v, 5 mL) was added to stop the enzymatic digestion. Samples were then centrifuged at 11000 rpm for 15 min. The residue was washed with ethanol (50 mL, 85%) and it was centrifuged again. The resulting residue was scooped out, oven dried at 100 ˚C to a constant weight. In each case, a blank starch without enzymatic hydrolysis was included to correct for initial concentration of soluble sugars. Starch digestibility was expressed as percent weight loss after α-amylase digestion.

***In vivo* digestibility**

Broilers were used in this study as a model for determining dry matter, starch, crude protein, true protein and gross energy digestibilities of untreated and treated samples. The experimental (Table 1) diets were given to their respective. The experiment was carried out with 3 day adaptation period, 2 days starvation for depleting digestive tract, then 1 day feeding and following 2 days starvation for complete excretion of undigested material. The samples of dropping avoided during final 72 h period were collected, weighted and frozen (-18 ˚C). Analyses of dry matter, starch, crude protein, true protein and gross energy of untreated and treated samples were conducted and calculations were carried out.

Table 1 Composition of the experimental diets

Component	Amount (g/kg diet)
Bitter vetch seed	108
Wheat grain	700
Corn grain	60
Soybean meal	110
Calcium Phosphate	3.30
Salt	2.50
Vitamin and mineral premix[a]	12.50
L-lysine HCl	2.50
L-threonine	1.20
GE (MJ/kg)	17.80

[a] Content per g of premix: Phosphorus: 0.1 g; Magnesium: 0.017 g; Calcium: 0.152 g; Sodium: 0.030 g; Retinol: 150 IU; Cholecalciferol: 30 IU; α-tocopherol acetate: 0.2 IU; Copper (as copper sulphate): 0.012 mg and Selenium (as selenium BCP): 3.2 µg.
GE: gross energy.

Statistical analysis

Treatments were analyzed as a completely randomized design under the general model:

$$Y_{ij} = \mu + Ti + e_{ij}$$

Where:
Y_{ij}: dependent variable.
µ: general mean.
T_i: treatmenti 1, 2, 3.
e_{ij}: experimental error.

Calculated using the GLM procedure of the SAS software (SAS, 2008). The broilers were the experimental units for all analyses. Treatment means were compared using the Duncan method, an α-value of 0.05 was used to assess significance and orthogonal polynomial contrast were performed to find a linear or quadratic response.

RESULTS AND DISCUSSION

Processing caused in significant (P<0.05) loss of moisture content in bitter vetch seeds (Table 2). The losses were: 2.3, 12.6, 19.2 and 23% for soaking, cooking, autoclaving and extruding, respectively. Treatments decreased the crude protein, crude fiber and starch contents of seeds. About 0.43% of crude protein, 0.19% of crude fiber and 0.47% of starch contents were lost on soaking Bitter vetch seeds in water.

About 3% of crude protein, 2.4% of crude fiber and 0.94% of starch contents were lost on cooking Bitter vetch seeds, 3.9% of protein, 4.3% of crude fiber and 5.45% of starch contents were lost when Bitter vetch seeds were cooked in an autoclave, and about 4.8% of protein, 10.1% of crude fiber and 9.5% of starch contents were lost on extruded Bitter vetch seeds. Ether extract was reduced on treatment but this reduce was not significant, while ash significantly increased on treatments (P<0.05).

The results of analysis of anti-nutritional factors in the raw seeds of bitter vetch were given in Table 3. On the basis of these results, the ranks of treatments from lowest to highest anti-nutritional factors were: 1) extruding, 2) autoclaving, 3) cooking and 4) soaking.

In Table 4, the enzymatic digestibility of Bitter vetch starch and its hydrothermal treatment derivatives are depicted. Starch digestibility of the raw Bitter vetch was between 17-32% during 10-26 hours incubation which became 57-68% after ordinary cooking, 66-81% after autoclaving, and 70-85% after extrusion. In fact, ordinary cooking, autoclaving and extrusion treatments improved starch digestibility by 112-235%, 165-311% and also 165-311% during incubation time, respectively.

The results of *in vivo* digestibility of untreated and hydrothermally treated Bitter vetch seeds are shown in Table 5. Digestibility of starch, dry matter, crude protein, true protein and gross energy increased significantly compared to control that followed the order: raw < soaking < cooking < autoclaving < extrusion. No data were found by authors regarding the effect of hydrothermal treatment on *in vivo* digestibility of Bitter vetch. On the basis of results, it is concluded that protein digestibility was improved by 2.3% for CP and 1.4% for true protein (TP) after soaking the Bitter vetch seed in distilled water, by 8.3% for CP and 5.8% for TP after cooking the Bitter vetch, and by 12.3% for CP and 9.7% for TP after autoclaving, whereas extrusion caused maximum improvement in protein digestibility, by 21.6% for CP and 18.6% for TP. On the basis of results, it is concluded that starch digestibility was improved by 2% after soaking the Bitter vetch seed in water, by 8.9% after cooking the Bitter vetch seed, and by 16.3% after autoclaving, whereas extrusion caused maximum improvement in starch digestibility, by 24.3%.

Low moisture content will be advantageous in maintenance and improvement of shelf life. The high protein content in bitter vetch seeds emphasizes their value as a vital source of nutrients. The losses in protein could be attributed to partial removal of certain amino acids, along with other nitrogenous compounds, on heating as has already been reported by other workers (Rehman and shah, 2005). It would be interesting to determine total, soluble and insoluble dietary fiber fractions in raw and treated bitter vetch seeds, to gain a better insight into the fiber contents. Losses in starch contents could be the result of solubilization of starch from bitter vetch seeds during heat treatments. The quantity of ash in any seed sample assumes importance, as it determines the nutritionally important minerals. Bitter vetch seeds contained a high amount of nitrogen free extract, which might be due to low lipid content. However, heat treatments increased the nitrogen free extract. Gopalan *et al*. (1989) reported that the increase in nitrogen free extracts might be attributed to heat-induced breakdown of complex sugars (polysaccharides) into simple extractable forms (e.g., free sugars). The highest impediment to consume any wild or under-utilized seeds is the presence of anti-nutritional factors, particularly those which are heat-stable and difficult to eliminate on processing. These anti-nutritional factors decrease the digestibility and bioavailability of nutrients in the intestine. Unprocessed legume proteins have been reported to have low nutritive value which was attributed to low protein digestibility and the presence of anti-nutritional factors (Pham and del Rosario, 1987). Protease inhibitors, tannin and other anti-nutritional factors have been suggested as factors responsible for the low digestibility of plant proteins (Pham and del Rosario, 1987). However, application of hydrothermally treatments could significantly decrease anti-nutritional factors and improve nutritive value of wild legume. The high content of total free phenolics, tannins and condensed tannins is known to inhibit the activity of the digestive enzymes and, thus, interfere with the digestion and absorption of nutrients. They may also cause damage to the mucosa of the digestive tract. Although the effects of hydrothermal treatments on phenolics and tannin contents of some materials have been reported, there is no information available in literature on the effect of hydrothermal treatment on tannin contents of bitter vetch. Since the phenolic compounds are water-soluble, it seems that the reduction in total free phenolic, tannin and condensed tannin level during soaking and cooking in the present study might be due to either the increased leaching out of phenolic substances or degradation by hydrothermal treatment.

Results achieved from autoclaving are in agreement with the previous report in legume seeds (Vijayakumari *et al*. 2007). The loss of phenolics due to autoclaving may be due to degradation or interaction with other components of seeds, such as proteins, to form insoluble complexes (Rehman and shah, 2005). It has been reported that extrusion cooking as a heat treatment affects and alters the nature of many food constituents including starches and proteins by changing their physical, chemical and nutritional properties (Iwe *et al*. 2004). Alonso *et al*. (2000) studied the effects of extrusion and conventional processing methods on protein and anti-nutritional factors in peas and they found varietal changes in their tannin contents, and extrusion was most effective in reducing tannins than the other processes. The potent anti-metabolic properties of canavanine result primarily from its ability to function as a highly effective antagonist of arginine metabolism due to its structural similarity to this protein amino acid. It is also believed to function in maintaining nitrogen requirements of developing plants and to contribute significantly to plant chemical defense.

Table 2 Chemical composition of processed bitter vetch seed (as g/100 g dry matter)

Treatment[2]	Moisture	Starch	CP	EE	CF	Ash	NFE
Unprocessed seeds (control)	6.07^{a}	42.21^{a}	22.80^{a}	3.02	5.23^{a}	5.95^{d}	56.93^{b}
Water soaking	5.93^{a}	42.11^{a}	22.70^{a}	3.02	5.22^{a}	6.00^{d}	57.13^{b}
Water soaking + cooking	5.31^{b}	41.82^{b}	22.10^{b}	3.00	5.10^{ab}	6.20^{c}	58.30^{a}
Water soaking + autoclaving	4.91^{c}	39.90^{c}	21.90^{c}	2.95	5.00^{b}	6.60^{b}	58.65^{a}
Water soaking + extrusion	4.67^{c}	38.80^{d}	21.70^{d}	2.90	4.70^{c}	6.85^{a}	59.18^{a}
SEM	0.42	0.20	0.50	0.20	0.60	0.30	0.03
P-value	0.70	0.54	0.61	0.80	0.78	0.69	0.80

CP: crude Protein; EE: ether extract; CF: crude fiber and NFE: nitrogen free extract.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effect of hydrothermal and extrusion processing on anti-nutritional factors contents in the bitter vetch seeds

Treatment	Total phenols (mg/100 g DM)	Tannin (mg/100 g DM)	Condensed tannin (mg/100 g DM)	Canavanine (mg/100 g DM)	Trypsin inhibitor (mg/100 g DM)
Unprocessed seeds (control)	202.1^{a}	188.3^{a}	230.2^{a}	78.5^{a}	2.0^{a}
Water soaking	189.3^{a}	176.5^{a}	219.4^{a}	69.6^{a}	1.8^{a}
Water soaking + cooking	147.2^{b}	155.1^{b}	198.1^{b}	55.4^{b}	0.8^{b}
Water soaking + autoclaving	74.5^{c}	95.6^{c}	151.4^{c}	48.3^{c}	ND
Water soaking + extrusion	35.6^{d}	79.4^{d}	114.6^{d}	39.2^{d}	ND
SEM	0.5	0.6	0.4	0.2	0.3
P-value	0.60	0.61	0.58	0.64	0.53

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
ND: not detectable.
SEM: standard error of the means.

Table 4 Time course of in vitro starch digestion (proportion of total starch) of the bitter vetch seeds

Treatment	Incubation time (hour)				
	10	14	18	22	26
Unprocessed seeds (control)	17.1^{e}	20.5^{e}	25.3^{d}	29.5^{e}	32.6^{d}
Water soaking	23.4^{d}	30.5^{d}	34.2^{c}	34.6^{d}	43.2^{c}
Water soaking + cooking	57.3^{c}	59.4^{c}	62.7^{b}	66.4^{c}	68.8^{b}
Water soaking + autoclaving	66.6^{b}	69.5^{b}	73.1^{a}	75.3^{b}	81.6^{a}
Water soaking + extrusion	93.7^{a}	73.9^{a}	78.5^{a}	81.2^{a}	85.3^{a}
SEM	0.1	0.5	0.4	0.6	0.7
P-value	0.03	0.04	0.51	0.03	0.55

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effects of processing on *in vivo* digestibilities of nutrients (%) in the bitter vetch seeds

Treatment	Starch	DM	GE	CP	TP
Unprocessed seeds (control)	43.5^{d}	61.4^{c}	37.8^{d}	76.2^{d}	81.4^{d}
Water soaking	44.3^{d}	62.8^{c}	38.9^{d}	78.1^{d}	82.6^{d}
Water soaking + cooking	47.4^{c}	66.8^{b}	41.5^{c}	82.6^{c}	86.5^{c}
Water soaking + autoclaving	50.6^{b}	70.3^{b}	46.1^{b}	85.6^{b}	89.4^{b}
Water soaking + extrusion	54.1^{a}	75.5^{a}	50.2^{a}	92.7^{a}	96.6^{a}
SEM	1.8	1.7	1.2	1.6	1.4
P-value	0.70	0.81	0.75	0.70	0.68

DM: dry matter; GE: gross energy; CP; crude protein and TP: true protein.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The arginine-like structure enables canavanine to bind many enzymes that usually interact with arginine, and it is incorporated into polypeptide chains, resulting in structurally aberrant canavanine-containing proteins (Adebowale *et al*. 2005). Based on the above observations, it may be stated that due to heat-stable nature of canavanine, hydrothermal treatments caused in only partially inactivating of this factor. Certain reports suggested that inactivation of heat-sensitive factors may not always be complete.

Trypsin inhibitors, due to their heat-sensitive nature were significantly reduced to undetectable amounts by the heating processes (autoclaving and extrusion). The results of this study were consistent with those mentioned by previous investigators that hydrothermal treatment reduced trypsin inhibitor activity (Rehman and shah 2005; Vijayakumari *et al*. 2007). Starches in legumes are particularly well protected from the polar environment of luminal fluids, and may not have access to α-amylase in the intestinal lumen

unless they have been physically altered. In addition, the digestibility of starch is generally inversely proportional to its amylose content (legume seeds contain high amylose starches), because the amylase action begins in the amorphous regions which fits with the generally held view that amylose represents amorphous starch (at least in normal and waxy genotypes) (Yu and Wang, 2007). Improvement in starch digestibility could be attributed to changes of microstructural of starch granules and hydrolysis of starch as a result of heat treatments. Several reasons were offered for the alteration of microstructure of starch granules; for example increased starch gelatinization, increased enzyme susceptibility, reduced starch resistant content and reduced amylose lipid complexes (Amornthewaphat *et al*. 2008). When starch molecules are heated in excess water, the crystalline structure is disrupted and water molecules become linked by hydrogen bonding to the exposed hydroxyl groups of amylose and amylopectin, which causes an increase in granule swelling and solubility. However, earlier workers also reported that hydrothermal treatment improves the digestibility of starch through destruction of anti-nutrients (Rehman and shah, 2005).

In fact, partial removal of anti-nutrients probably created a large space within the matrix, which increased the susceptibility to enzymatic attack and consequently improved the digestibility of starch after the heat process. Also, the increase in digestibility of starch after extrusion may be explained on the basis that the starch granules lose their structural integrity due to increased shearing action and kneading in the extruder barrel which ultimately decrease the size distribution of the granule and increase their susceptibility towards enzymatic attack. Somewhat low values of digestibilities from the extrusion cooked starch or starchy foods have also been seen sometimes which may be attributed to the formation of amylose-lipid complexes, starch-protein interaction, and limited water availability which prolongs the starch digestibility during enzymatic hydrolysis. Results mentioned above are in agreement with the previous report in legume seeds (Htoon *et al*. 2009; Amornthewaphat *et al*. 2010).

Changes in starch properties during extrusion may also be reflected in the responses of animals fed extruded diets compared to raw diets (Amornthewaphat *et al*. 2010). Vijayakumari *et al*. (2007) attributed the poor nutritive value of legumes to the presence of some forms of proteins which inhibit the digestive enzymes such as trypsin and chymotrypsin inhibitors.

Therefore, higher protein digestibility after heat treatment may be due to the reduction or elimination of different anti-nutrients. Tannin, condensed tannins and trypsin inhibitors, are known to interact with protein to form complexes. This interaction increases the degree of cross-linking, decreasing the solubility of proteins and making protein complexes less susceptible to proteolytic attack than the same protein alone (Vijayakumari *et al*. 2007). Due to their hydroxyl groups, tannins may interact with and form complexes with proteins, which may lead to precipitation because of the large size of the tannins.

Study of Adebowale *et al*. (2005) showed that the tannins may also exert steric effects (due to their large size) and prevent enzymes access to the proteins. So it seems that partial removal of tannin probably created a large space within the matrix, which increased the susceptibility to enzymatic attack and consequently improve the digestibility of protein. Results obtained in this experiment were consistent by several authors that determined hydrothermal treatment improved *in vivo* digestibility of starch (Amornthewaphat and Attamangkune, 2008).

CONCLUSION

Hydrothermal processing has been used as a means to inactivate anti-nutritional factors and increase of nutritional quality of bitter vetch seeds. Maximum improvement in protein quality (i.e. *in vivo* crude and true protein digestibility) was observed at the extrusion processing. However, future works should give priority to decomposition of the amino acid by long soak at temperatures above 184 ˚C or maybe the extrusion processing or interacting each other. In this paper it has been shown that the hydrothermal processing improved *in vivo* and *in vitro* digestibility of starch values. Hydrothermal processing decreased tannin, canavanine and trypsin inhibitor activity of bitter vetch seeds. Further studies are needed to evaluate the definite effect of hydrothermal processing on anti-nutritional factors using pure molecules. Also it remains to be further clarified what happen if we increase *Vicia ervilia* levels in diet.

ACKNOWLEDGEMENT

Authors are grateful to Nuclear Science and Technology School, Atomic Energy Organization of Iran for the operations. This paper was elicited from PhD thesis of second author (Tehran Science and Research Branch, Islamic Azad University). The authors are grateful to the Islamic Azad University for research funding support. We also thank all staffs in the poultry unit of AEOI, for the assistance in the care and feeding of the chicks used in this research.

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