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

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Abstract: Comparative effects of extrusion cooking and conventional processing methods (cooking and autoclaving) on physicochemical properties, anti-nutritional factors and subsequent effects on digestibility [Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS), enzyme-Resistant Starch (RS), *in vitro* and *in vivo* digestibility of starch] 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. The results showed that Apparent Amylose Content (AAC) was significantly decreased ($p<0.05$). Treatments of seeds resulted in significant reduction of total phenols, tannins, condensed tannins, canavanine and trypsin inhibitor activity ($p<0.05$). Conventional and extrusion processing decreased the proportions of SDS and RS while increased the proportion of RDS ($p<0.05$). 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: Conventional and extrusion processing, *V. ervilia*, anti-nutritional factors, digestibility, rapidly digestible, slowly digestible and resistant starch

INTRODUCTION

Bitter vetch (*Vicia ervilia*) is known for its high nutritional value, capacity of nitrogen fixation and ability to grow in poor soils and its seeds contain about 22.8% CP and 42.2% starch (Farran *et al*., 2001). *V. ervilia* 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 including L-canavanine (0.035-0.11%), trypsin inhibitor (2.14 mg g^{-1} DM) and tannin (2.01 g kg^{-1} DM) (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 versatility, 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).

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Therefore, in this study, it was aimed to evaluate effects of extrusion on physicochemical properties, 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

The experiment was performed at the experimental 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.

Collection of the seed sample: The seeds of *V. ervilia* were obtained from the agricultural and natural resources research institute, Sari, Mazandaran, Iran. Soon after collection, after removing 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: *V. ervilia* 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: *V. ervilia* seeds soaked at the same conditions mentioned earlier. 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 meta-bisulphite 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. Cages were 37 cm wide by 42 cm tall by 30 cm deep, contained a roost and were wire bottomed with provision for collection of excreta. Prior to the adaptation and trial period chicks were fed Chick Starter Crumb (Dodson and Horrell Ltd. Northamptonshire, UK: AME, 11.7 MJ kg^{-1}; the following in g/kg; CP, 190; Oil, 33; Fibre, 33 g; Ash, 51; Ca, 9; available P, 4.5; Lysine, 10). At day 19 the birds began an adaptation period where they

Table 1: Experimental diets composition

Components	Amount (g/kg diet)
Bitter vetch	108.00
Wheat	700.00
maize	60.00
Soya	110.00
Calcium phosphate	3.30
Vitamin and mineral premix[a]	12.50
Lysine	2.50
L-threonine	1.20
Salt	2.50
GE (MJ/kg)	17.86

[a]Content per g of premix: 0.1 g phosphorus, 0.017 g magnesium, 0.152 g calcium, 0.030 g sodium, 150 IU Retinol, 30 IU cholecalciferol, 0.2 IU α-tocopherol acetate, 0.012 mg copper (as copper sulphate), 3.2 μg selenium (as selenium BCP)

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. During the trial period, temperature was maintained at 21°C and the birds were kept under artificial light for 23 h per day with 1 h of dark. The air in the metabolism room was continuously circulated and humidity monitored.

Chemical composition: Moisture content was determined from the samples before and after they were stored overnight in an oven at 105°C (Methods 925.09; AOAC, 1995). Nitrogen was determined by using a Dosimat-776 Metrohm apparatus (Metrohm Co., Switzerland) according to AOAC (Method 984.13; 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 McCleary *et al.* (1994). Fat content was determined with a Solvent Extractor (Behr Labour-Technik, Dusseldorf, Germany) equipped with six Soxhlet posts (Method 920.39; AOAC, 1995). Ash was determined by burning duplicate 2 g samples at 540°C for 3 h in a muffle furnace (Method 942.05; 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 sub-sample and each sample was analyzed in duplicate.

Antinutritional features

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 (Burns, 1971). 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.*, 1985). 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. The 10 g 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 *V. ervilia* samples: The 2 g of a finely ground sample of *V. ervilia* 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 10,000×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: The 1 mL 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 0.005-0.08 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 solutioninstead 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 (20,000 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.

Englyst classification of starch: The digestibility of starch was analyzed according to the procedure of Englyst *et al.* (1992) with a slight modification. To prepare enzyme solution I, amyloglucosidase solution (0.14 mL) was diluted to 6.0 mL with deionized water. Enzyme solution II was prepared by suspending porcine pancreatic α-amylase (12.0 g) in water (80.0 mL) with magnetic stirring for 10 min, centrifuging the mixture for 10 min at 1500 g then transferring a portion (54.0 mL) of the supernatant into a beaker. Enzyme III was prepared immediately before use by mixing water (4.0 mL), enzyme solution I (6.0 mL) and enzyme solution II (54.0 mL). A starch sample (200 mg) was dissolved in phosphate buffer (15 mL, 0.2 mol L^{-1} and pH 5.2) by vortexing. After equilibrated at 37°C for 5 min, 7 glass balls (10 mm diameter) and enzyme solution III (5.0 mL) were then added followed by incubation in a water bath at 37°C with shaking (150 rpm). Aliquots of hydrolyzed solution (0.5 mL) were taken at different time intervals and mixed with 4 mL of absolute ethanol to deactivate the enzymes. The glucose content of the hydrolyzates was determined using glucose oxidase/peroxidase assay kits. Percentage of hydrolyzed starch was calculated by multiplying a factor of 0.9 with the glucose content. Each sample was analyzed in triplicate. The values of different starch fractions of RDS, SDS and RS were obtained by combining the values of G20 (glucose released after 20 min), G120 (glucose released after 120 min), FG (free glucose) and TG (total glucose) and using the following equation:

$$RDS\ (\%) = (G120\text{-}FG)\times 0.9\times 100$$

$$SDS\ (\%) = (G120\text{-}G20)\times 0.9\times 100$$

$$RS\ (\%) = (TG\text{-}FG)\times 0.9\times 100\text{-}(RDS+SDS)$$

***In vitro* digestibility:** Enzymatic digestibility by α-amylase was investigated for untreated and treated *V. ervilia* 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^{-1}, Fluka) were added. About 5 replicate preparations were made for each samplein 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 11,000 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 days 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.

Apparent and absolute amylose content: Apparent and absolute amylose content of starch samples was determined by the method given by Williams *et al.* (1970) and the analysis was repeated five times. Milled *V. ervilia* seeds were ground into flour and then (20 mg db) were dispersed in KOH (0.5M) and made up to 100 mL using distilled water. To an aliquot (10 mL) of the solution, 5 mL of HCl (0.1M) and 0.5 mL of iodine reagent (0.1%) were added, diluted to 50 mL. The amylose present in the *V. ervilia* seeds forms a complex with the iodine. The color change (measured using a spectrophotometer at 625 nm) in the solution was correlated to the amount of the iodine-amylose complex that was formed. Apparent amylose content was derived from a standard curve using amylose and amylopectin blends. Iodine affinity of 20% for amylose was used for calculation (Takeda and Hizukuri, 1987). The iodine affinity of pure amylopectin was determined following the same method. Absolute amylose contents were determined by subtracting the iodine affinities of amylopectins from that of the defatted whole starches following the method of Takeda and Hizukuri (1987).

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

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

Where:

Y_{ij} = The dependent variable
μ = The general mean
T_i = The treatment i = 1, 2, 3
e_{ij} = The experimental error

alculated 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

Proximal features: Treatments caused in significant loss of moisture ($p<0.05$) (Table 2). About 2.3% of moisture content was lost on soaking *V. ervilia* seed in distilled water, 12.68% of moisture content was lost on cooking *V. ervilia*, 19.27% of moisture content was lost when *V. ervilia* seeds were cooked in an autoclave and about 23% of moisture was lost on extruded *V. ervilia* seeds. 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 *V. ervilia* seeds in water. About 3% of crude protein, 2.4% of crude fiber and 0.94% of starch contents were lost on cooking *V. ervilia* seeds, 3.94% of protein, 4.39% of crude fiber and 5.45% of starch contents were lost when *V. ervilia* seeds were cooked in an autoclave and about 4.82% of protein, 10.13% of crude fiber and 9.52% of starch contents were lost on extruded *V. ervilia* seeds. Ether extract was reduced on treatment but this reduce was not significant while ash significantly increased on treatments ($p<0.05$).

Anti-nutritional features: The results of analysis of anti-nutritional factors in the raw seeds of *V. ervilia* were given in Table 3. In this study, results showed that extrusion treatment caused the highest decrease in anti-nutritional factors (total phenols = 82%; tannin = 58%; condensed tannin = 50.4%; canavanine = 50%; trypsin inhibitor = 100%) while the *V. ervilia* treated by autoclaving showed the second highest decrease (total phenols = 63%; tannin = 49%; condensed tannin = 34%; canavanine = 38.46%; trypsin inhibitor = 100%) and cooking showed third highest decrease (total phenols = 27%; tannin = 17%; condensed tannin = 14%; canavanine = 29.48%; trypsin inhibitor = 57%). Sadeghi *et al.* (2004) had reported that *V. ervilia* seeds

Table 2: Chemical composition of processed *V. ervilia* seed (as g/100 g dry matter)

Treatments	Moisture	Red[a] (%)	Starch	Red[a] (%)	Crude protein	Red[a] (%)	Ether extract	Red[a] (%)	Crude fiber	Red[a] (%)	Ash	Incr[b] (%)	NFE	Incr[b] (%)
Unprocessed seeds (control)	6.07^{a}	-	42.20^{a}	-	22.8^{a}	-	3.02	-	5.23^{a}	-	5.95^{d}	-	56.93^{b}	-
Water soaking	5.93^{a}	2.30	42.00^{a}	0.47	22.7^{a}	0.43	3.02	0.00	5.22^{a}	0.19	6.00^{d}	0.84	57.13^{b}	0.35
Water soaking+cooking	5.30^{b}	12.68	41.80^{b}	0.94	22.1^{b}	3.00	3.00	0.66	5.10^{ab}	2.40	6.20^{c}	4.20	58.30^{a}	2.40
Water soaking+autoclaving	4.90^{c}	19.27	39.90^{c}	5.40	21.9^{c}	3.90	2.95	2.30	5.00^{b}	4.39	6.60^{b}	10.90	58.65^{a}	3.00
Water soaking+extrusion	4.67^{c}	23.00	38.80^{d}	8.00	21.7^{d}	4.80	2.90	3.90	4.70^{c}	10.00	6.85^{a}	15.00	59.18^{a}	3.90
SEM	0.40	-	0.02	-	0.5	-	0.20	-	0.60	-	0.30	-	0.03	-

Values followed by the different superscripts letter within a column differ significantly ($p<0.05$) from each other; SEM: Standard Error of the Means; [a]Reduction indicates % decrease over raw value; [b]Increment indicates % increase over raw values

contained 0.035-0.11% (mean: 0.083) canavanine which is quiet comparable to the levels detected in the present study (Table 3).

Englyst classification of starch: Starch nutritional fractions (RDS, SDS and RS) of hydrothermally treated *V. ervilia* seeds starches are presented in Table 4. RDS, SDS and RS levels followed the order: extrusion >autoclaving>cooking>soaking; soaking>cooking>autoclaving>extrusion and soaking> cooking>autoclaving>extrusion, respectively. The reduction of RDS content was 98, 663, 736 and 810%; SDS content reduced 31.27, 74.27, 87.57 and 97.97% and RS content decreased 9.98, 77.31, 82.75 and 90.01% for soaked, cooked, autoclaved and extruded *V. ervilia* seeds, respectively.

***In vitro* starch digestibility:** In Fig. 1, the enzymatic digestibility of *V. ervilia* starch and its hydrothermal treatment derivatives are depicted. Starch digestibility of the raw *V. ervilia* was between 17-32% during 10-26 h 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.

***In vivo* digestibility:** The results of *in vivo* digestibility of untreated and hydrothermally treated *V. ervilia* seeds are shown in Table 5. Digestibility of starch, dry matter, crude protein, true protein and gross energy increased

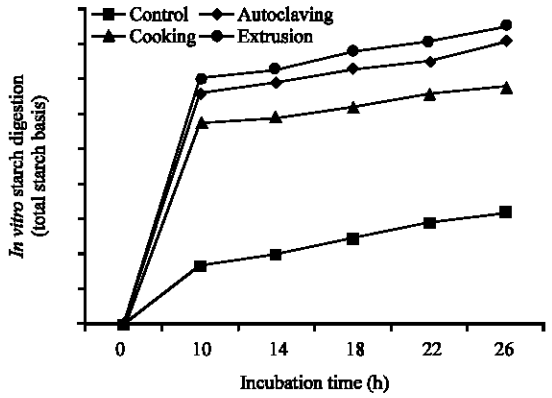

Fig. 1: Time course of *in vitro* starch digestion (proportion of total starch) of *V. ervilia* seed

Table 3: Effect of hydrothermal and extrusion processing on anti-nutritional factors contents in the *V. ervilia* seeds

Treatments	Total phenols (mg/100 g) (d.m.)	Reduction[a] (%)	Tannin (mg/100 g) (d.m.)	Reduction[a] (%)	Condensed tannin (mg/100 g) (d.m.)	Reduction[a] (%)	Canavanine (mg/100 g) (d.m.)	Reduction[a] (%)	Tryspin inhibitor (mg/100 g) (d.m.)	Reduction[a] (%)
Unprocessed seeds (control)	202.0^{a}	-	188.00^{a}	-	230.00^{a}	-	78.0^{a}	-	2.03^{a}	-
Water soaking	189.0^{a}	6.4	176.00^{a}	6.3	219.00^{a}	4.8	69.0^{a}	11.53	1.86^{a}	8.37
Water soaking+cooking	147.0^{b}	27.0	155.00^{b}	17.0	198.00^{b}	14.0	55.0^{b}	29.48	0.87^{b}	57.00
Water soaking+autoclaving	74.0^{c}	63.0	95.00^{c}	49.0	151.00^{c}	34.0	48.0^{c}	38.46	ND	100.00
Water soaking+extrusion	35.0^{d}	82.0	79.00^{d}	58.0	114.00^{d}	50.4	39.0^{d}	50.00	ND	100.00
SEM	0.5	-	0.69	-	0.45	-	0.2	-	0.3	-

ND = Not Detectable; Means not sharing a common superscript letter with in a column are significantly different ($p<0.05$); [a]Reduction indicates % decrease over raw value

Table 4: The amounts of Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS) and Resistant Starch (RS) of processed *V. ervilia* seed starches

Treatments	RDS (%)	Increment[a] (%)	SDS (%)	Reduction[b] (%)	RS (%)	Reduction[b] (%)
Unprocessed seeds (control)	10.3^{d}	-	34.6^{a}	-	55.1^{a}	-
Water soaking	20.4^{d}	98	30.0^{b}	31.27	49.6^{b}	9.98
Water soaking+cooking	78.6^{c}	663	8.9^{c}	74.27	12.5^{c}	77.31
Water soaking+autoclaving	86.2^{b}	736	4.3^{d}	87.57	9.5^{d}	82.75
Water soaking+extrusion	93.8^{a}	810	0.7^{e}	97.97	5.5^{e}	90.01
SEM	0.2	-	0.4	-	0.6	-

Means with the same superscript letters with in a column are not significantly different at $p<0.05$ level; [a]Reduction indicate % decrease over raw values; [b]Increment indicate % increase over raw values

Table 5: Effects of processing on *V. ervilia* seed *in vivo* digestibility (%)

Treatments	Starch	Increment[a] (%)	Dry matter	Increment[a] (%)	Gross energy	Increment[a] (%)	Crude protein	Increment[a] (%)	True protein	Increment[a] (%)
Unprocessed seeds (control)	43.50^{d}	-	61.42^{c}	-	37.80^{d}	-	76.22^{d}	-	81.43^{d}	-
Water soaking	44.39^{d}	2.04	62.87^{c}	2.36	38.92^{d}	2.96	78.00^{d}	2.33	82.65	1.49
Water soaking+cooking	47.40^{c}	8.96	66.80^{b}	8.75	41.50^{c}	9.78	82.60^{c}	8.37	86.50^{c}	6.22
Water soaking+autoclaving	50.60^{b}	16.32	70.30^{b}	14.45	46.10^{b}	21.95	85.60^{b}	12.30	89.40^{b}	9.78
Water soaking+extrusion	54.10^{a}	24.36	75.50^{a}	22.92	50.20^{a}	32.80	92.70^{a}	21.62	96.60^{a}	18.62
SEM	0.60	-	0.30	-	0.02	-	0.40	-	0.07	-

Values followed by the different superscripts letter within a column differ significantly ($p<0.05$) from each other; SEM: Standard Error of the Means; [a]Increment indicate % increase over raw values

Table 6: Amylose contents of *V. ervilia* starches

	Iodine affinity (%)[a]		Amylose content (%)					
Treatments	Starch	Reduction[c] (%)	Amylopectin	Reduction[c] (%)	Apparent[b]	Reduction[c] (%)	Absolute[d]	Reduction[c] (%)
Unprocessed seeds (control)	8.70[a]	-	1.10[a]	-	43.5[a]	-	40.21[a]	-
Water soaking	8.60	1.14	1.10[a]	0.00	43.0[a]	1.14	39.68[a]	1.31
Water soaking+cooking	8.30[ab]	4.50	1.00[a]	9.00	37.3[b]	14.25	38.40[b]	4.50
Water soaking+autoclaving	8.00[b]	8.00	0.90[ab]	18.10	34.2[c]	21.37	37.10[c]	7.73
Water soaking+extrusion	7.40[c]	14.90	0.80[b]	27.27	30.8[d]	29.19	34.30[d]	14.69
SEM	0.60	-	0.20	-	0.5	-	0.04	-

[a]Averaged from at least two analyses. [b]Apparent amylose content (%) = $IA_{starch}/IA_{Amylose}\times100$. Iodine affinity for pure amylose was 20%; [c]Reduction indicate % decrease over raw values; [d]Absolute amylose content (%) = $(IA_{starch}-IA_{amylopectin})/(IA_{amylose}-IA_{amylopectin})\times100$. Values followed by the different superscripts letter within a column differ significantly ($p<0.05$) from each other

significantly compared to control that followed the order: Raw<soaking<Cooking<Autoclaving<Extrusion. No data were found by researchers regarding the effect of hydrothermal treatment on *in vivo* digestibility of *V. ervilia*. On the basis of results it is concluded that protein digestibility was improved by 2.33% for CP and 1.49% for TP after soaking the *V. ervilia* seed in distilled water by 8.37% for CP and 5.85% for TP after cooking the *V. ervilia* and by 12.3% for CP and 9.78% for TP after autoclaving whereas extrusion caused maximum improvement in protein digestibility by 21.62% for CP and 18.62% for TP. On the basis of results, it is concluded that starch digestibility was improved by 2.04% after soaking the *V. ervilia* seed in water by 8.96% after cooking the *V. ervilia* seed and by 16.32% after autoclaving whereas extrusion caused maximum improvement in starch digestibility by 24.36%.

Apparent and absolute amylose content: Both apparent and absolute amylose contents of the granule starch of *V. ervilia* were analyzed and the results are shown in Table 6. Apparent amylose contents were reduced by 1.14, 4.59, 6.89 and 11.49% and absolute amylose content reduced by 1.31, 5.27, 7.08 and 11.46% by soaking, cooking, autoclaving and extrusion, respectively. This effect of hydrothermal pretreatment on AAC was associated with the structure of starch. The values of the apparent amylose contents were larger than that of the absolute amylose content.

DISCUSSION

Proximal features: Low moisture content will be advantageous in maintenance and improvement of shelf life. The high protein content in *V. ervilia* 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 researchers (Rehman and Shah, 2005). It would be interesting to determine total, soluble and insoluble dietary fiber fractions in raw and treated *V. ervilia* seeds, to gain a better insight into the fiber contents. Losses in starch contents could be the result of solubilization of soluble starch from *V. ervilia* seeds during heat treatments. The quantity of ash in any seed sample assumes importance as it determines the nutritionally important minerals. *V. ervilia* 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. Narasinga-Rao *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 (free sugars).

Anti-nutritional features: 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. 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.

Phenolics and tannins: 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 *V. ervilia*. 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 earlier 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.

Canavanine: 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 defence. 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.

Trypsin inhibitor activity: 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 earlier investigators that hydrothermal treatment reduced trypsin inhibitor activity (Rehman and Shah, 2005; Vijayakumari *et al*., 2007).

Englyst classification of starch: The large amounts of RS in the raw *V. ervilia* diet reflect a high content of starch granules with a C-type crystal structure and a high amylose to amylopectin ratio (Zhang *et al*., 2008), along with physical entrapment of the starch within fibrous, thick-walled parenchyma cells (Zhang *et al*., 2008). Heat and moisture are two of the most important factors that affect the gelatinization and retrogradation of starch. The increases in RDS levels and the decrease in RS levels on cooking and autoclaving reflect disruption of some of the double helices forming the starch crystallites. During gelatinization, the inter and intra-molecular hydrogen bonds between starch chains are disrupted and most swollen starch granules are completely disrupted by excess heat and mechanical agitation resulting in transformation to a continuous amorphous structure which allows physical accessibility to enzymes (Chung *et al*., 2009).

Although, a significant number of studies have been carried out on the effect of extrusion on classification of starch, the results are mixed. Some studies showed that extrusion decreases RS content (Chanvrier *et al*., 2007; Htoon *et al*., 2009). On the other hand, other investigators have reported a higher yield of RS (Agustiniano-Osornio *et al*., 2005). Severe heat treatments including extrusion cooking may lead to formation of resistant starch due to intimate packing of amylose upon cooling (Haralampu, 2000) but shearing action of the extruder screw in the presence of high temperature may have caused degradation of longer amylose chains into small molecular fragments that could not be incorporated into a crystalline structure upon cooling (Haralampu, 2000). The fragmented starches may have remained more or less in an amorphous condition. Also, the rapid drying that occurs after extrusion might not have resulted in the opportunity for extensive retrogradation to occur. Each of these factors could be responsible for lower RS values in extruded starch. In agreement with current observations, Chanvrier *et al*. (2007) also, reported a marked increase in the RDS fraction, accompanied by a significant reduction of RS and SDS after extrusion.

***In vitro* starch digestibility:** 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 gelatinizationincreased enzyme susceptibility, reduced starch resistant content and reduced amylose lipid complexes (Amornthewaphat and Attamangkune, 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 researchers 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. 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 complexation, starch-protein interaction and limited water availability which prolongs the starch digestibility during enzymatic hydrolysis.

***In vivo* digestibility:** 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 researchers that determined hydrothermal treatment improved *in vivo* digestibility of starch (Amornthewaphat and Attamangkune, 2008; Li *et al.*, 2010).

Apparent and absolute amylose content: Amylose is an essentially linear polymer of α-(1-4)-linked-D-glucopyranosyl units with up to 0.1% α-(1-6) linkages with Degree of Polymerization (DP_n) of 800-4920. Amylopectin consist of α-(1-4)-linked-D-glucosyl chains and is highly branched with 5-6% α-(1-6)-bonds with degree of polymerisation of 8200-12,800. Evidently, the content and degree of polymerization of amylopectin were so higher than that of amylose. So, the amylopectin had higher probability to be broken and cleaved (Yu and Wang, 2007). These information is consistent with results achieved from current study in which hydrothermal treatment caused in reduce of apparent amylose content and increase of starch digestibility. Lan *et al.* (2008) claimed that decreased apparent amylose content on hydrothermal treatment could be associated with a decrease in the number of helical turns as a result of a change in amylose conformation and restriction in the ability of amylose to form longer or more ordered helical segments by Amylose-Amylose (AM-AM) and/or Amylose-Amylopectin (AM-AMP) interactions formed on hydrothermal treatment thereby decreasing the color intensity of the amylose-iodine complex.

CONCLUSION

Hydrothermal processing has been used as a means to inactivate anti-nutritional factors and increase of nutritional quality of *V. ervilia* seeds. The present research clearly shows that hydrothermal processing of *V. ervilia* can improve its nutritional quality. Maximum improvement in protein quality (i.e., *in vivo* protein digestibility) was observed at the extrusion processing. Hydrothermal processing decreased tannin, canavanine and trypsin inhibitor activity (approximately 50.9 and 83% reduction, respectively) of *V. ervilia* seeds. Hydrothermal processing induced the degradation of amylose and amylopectin and consequently altered the physicochemical properties including a decrease in apparent amylose content. Further studies are needed to evaluate the definite effect of hydrothermal processing on anti-nutritional factors using pure molecules.

REFERENCES

AOAC, 1995. Official Methods of Analysis. 16th Edn., Association of Official Analytical Chemists, Washington, DC., USA.

Adebowale, Y.A., I.A. Adeyemi and A.A. Oshodi, 2005. Variability in the physicochemical, nutritional and antinutritional attributes of six *Mucuna* species. Food Chem., 89: 37-48.

Agustiniano-Osornio, J.C., R.A. Gonzalez-Soto, E. Flores-Huicochea, N. Manrique-Quevedo, L. Sanchez-Hernandez and L.A. Bello-Perez, 2005. Resistant starch production from mango starch using a single-screw extruder. J. Sci. Food Agric., 85: 2105-2110.

Aletor, V.A., A.V. Goodchild and A.M. Abd El Moneim, 1994. Nutritional and antinutritional characteristics of selected *Vicia* genotypes. Anim. Feed Sci. Technol., 47: 125-139.

Alonso, A., G. Grant, P. Dewey and F. Marzo, 2000. Nutritional assessment *in vitro* and *in vivo* of raw and extruded peas (*Pisum sativum* L.). J. Agric. Food Chem., 48: 2286-2290.

Amornthewaphat, N. and S. Attamangkune, 2008. Extrusion and animal performance effects of extruded maize quality on digestibility and growth performance in rats and nursery pigs. Anim. Feed Sci. Technol., 144: 292-305.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Burns, R.E., 1971. Method for estimation of tannin in grain Sorghum. Agron. J., 63: 511-512.

Cacho, J., M.A. Garcia and I. Ferrando, 1989. Selective spectrophotometric determination of canavanine. Analyst, 114: 965-968.

Chanvrier, H., S. Uthayakumaran, I.A.M. Appelqvist, M.J. Gidley, E.P. Gilbert and A. Lopez-Rubio, 2007. Influence of storage conditions on the structure, thermal behavior and formation of enzyme-resistant starch in extruded starches. J. Agric. Food Chem., 55: 9883-9890.

Chung, H.J., Q. Liu and R. Hoover, 2009. Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches. Carbohydrate Polym., 75: 436-447.

Englyst, H.N., S.M. Kingman and J.H. Cummings, 1992. Classification and measurement of nutritionally important starch fractions. Eur. J. Clin. Nutr., 46: S33-S50.

Farran, M.T., G.W. Barbour, M.G. Uwayjan and V.M. Ashkarian, 2001. Metabolizable energy values and amino acid availability of vetch (Vicia sativa) and Ervil (*Vicia ervilia*) seeds soaked in water and acetic acid. Poult. Sci., 80: 931-936.

Haralampu, S.G., 2000. Resistant starch-a review of the physical properties and biological impact of RS_3. Carbohydr. Polym., 41: 285-292.

Htoon, A., A.K. Shrestha, B.M. Flanagan, A. Lopez-Rubio, A.R. Bird, E.P. Gilbert and M.J. Gidley, 2009. Effects of processing high amylose maize starches under controlled conditions on structural organisation and amylase digestibility. Carbohydr. Polym., 75: 236-245.

Iwe, M.O., D.J. Van Zuilichem, W. Stolp and P.O. Ngoddy, 2004. Effect of extrusion cooking of soy-sweet potato blends on available lysine content and browning index of extrudates. J. Food Eng., 62: 143-150.

Lan, H., R. Hoover, L. Jayakody, Q. Liu and E. Donner *et al*., 2008. Impact of annealing on the molecular structure and physicochemical properties of normal, waxy and high amylose bread wheat starches. Food Chem., 111: 663-675.

Li, Y., Z. Fang, J. Dai, G. Partridge, Y. Ru and J. Peng, 2010. Corn extrusion and enzyme addition improves digestibility of corn/soy based diets by pigs: *In vitro* and *in vivo* studies. Anim. Feed Sci. Technol., 158: 146-154.

McCleary, B.V., V. Solah and T.S. Gibson, 1994. Quantitative measurement of total starch in cereal flours and products. J. Cereal Sci., 20: 51-58.

Narasinga Rao, B.S., Y.G. Deosthale and K.C. Pant, 1989. Nutritive Value of Indian Foods. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India, pp: 47-91.

Pham, C.B. and R.R. Del Rosario, 1987. Inactivation of trypsin inhibitors using the extrusion process. Proceedings of the 7th World Congress of Food Science and Technology, September 28-October 2, 1987, Singapore, pp: 359-366.

Porter, L.J., L.N. Hrstich and B.G. Chan, 1985. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry, 25: 223-230.

Rehman, Z. and W.H. Shah, 2005. Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. Food Chem., 91: 327-331.

Rosset, J., F. Barlocher and J.J. Oertli, 1982. Decomposition of conifer needles and deciduous leaves in two Black Forest and two Swiss Jura streams. Int. Rev. Gesamten Hydrobiol., 67: 695-711.

SAS., 2008. SAS Institute User"s Guide: Statistics. Version 9.2. SAS Institute, Cary, USA.

Sadeghi, G., A. Samie, J. Pourreza and H.R. Rahmani, 2004. Canavanine content and toxicity of raw and treated bitter Vetch (*Vicia ervilia*) seeds for broiler chicken. Int. J. Poult. Sci., 3: 522-529.

Smith, C., W. van Megan, L. Twaalfhoven and C. Hitchcock, 1980. The determination of trypsin inhibitor levels in foodstuffs. J. Sci. Food Agric., 31: 341-350.

Takeda, Y. and S. Hizukuri, 1987. Structures of rice amylopectins with low and high affinities for iodine. Carbohydrate Res., 168: 79-88.

Vijayakumari, K., M. Pugalenthi and V. Vadivel, 2007. Effect of soaking and hydrothermal processing methods on the levels of antinutrients and *in vitro* protein digestibility of *Bauhinia purpurea* L. seeds. Food Chem., 103: 968-975.

Williams, P.C., F.D. Kuzina and I. Hlynka, 1970. A rapid colorimetric procedure for estimating the amylose content of starches and flours. Cereal Chem., 47: 411-420.

Yu, Y. and J. Wang, 2007. Effect of g-ray irradiation on starch granule structure and physicochemical properties of rice. Food Res. Int., 40: 297-303.

Zhang, D.P., W.W. Collins and M. Andrade, 1995. Estimation of genetic variance of starch digestibility in sweetpotato. Hortscience, 30: 348-349.

Zhang, G., A.O. Zihua and B. Hamaker, 2008. Nutritional property of endosperm starches from maize mutants: A parabolic relationship between slowly digestible starch and amylopectin fine structure. J. Agric. Food Chem., 56: 4686-4694.