Radiation Physics and Chemistry 80 (2011) 1441–1447

Contents lists available at SciVerse ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Investigation of electron beam irradiation effects on anti-nutritional factors, chemical composition and digestion kinetics of whole cottonseed, soybean and canola seeds

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ARTICLE INFO

Article history:
Received 19 June 2011
Accepted 12 August 2011
Available online 22 August 2011

Keywords:
Oilseed
Electron beam irradiation
Glucosinolate
Trypsin inhibitor
Gossypol

ABSTRACT

This study was completed to determine effects of electron beam (EB) irradiation at doses of 15, 30 and 45 kGy on anti-nutritional factors, ruminal degradation and *in vitro* crude protein (CP) digestibility of whole cottonseed (WCS), soybean (SB) and canola seeds (CS). EB-irradiation eliminated completely ($P<0.001$) phytic acid of WCS, SB and CS at a dose of 30 kGy. EB-irradiation decreased linearly ($P<0.001$) the total glucosinolate content of CS. Trypsin inhibitor activity of 15, 30 and 45 kGy EB-irradiated SB was decreased by 19, 73 and 88%, respectively. Free gossypol content of WCS was reduced linearly ($P<0.001$) by irradiation. EB-irradiation increased linearly ($P<0.001$) CP digestibility of feeds. In conclusion, EB-irradiation was an effective processing method for improving the nutritive value of WCS, SB and CS.

1. Introduction

Whole cottonseed, soybean and canola seeds are readily available sources of energy, protein and other nutrients for high producing dairy cows and other animals. However, their proteins are extensively degraded in the rumen (NRC, 2001) and especially in monogastric the nutritional quality of plant proteins is affected by the presence of anti-nutritional factors such as proteinase inhibitors (Farag, 1998), phytic acid (Siddhuraju et al., 2002; Bhat et al., 2007), gossypol (Francis et al., 2001) and glucosinolate (Tripathi and Mishra, 2007).

Protease inhibitors in SB may cause an increase in the secretion of digestive enzymes, including trypsin, chymotrypsin and elastase by inducing hypertrophy and hyperplasia of the pancreas and reducing CP digestibility in monogastrics (Liener and Kakade, 1980; Siddhuraju et al., 2002). Phytic acid, a common constituent of plant tissues, chelates mineral cations and proteins, forming insoluble complexes, which leads to reduced bioavailability of trace minerals, reduced digestibility of proteins and inhibited proteolytic enzyme activity (Bitar and Reinhold, 1972; Duodu et al., 1999). Glucosinolates in CS are plant secondary metabolites that have long been of toxicological effects. Glucosinolates are biologically inactive, non-toxic and their adverse effects are only evident following hydrolysis. Glucosinolates are responsible for reducing feed intake, inducing iodine deficiency and depressing fertility in animals (Ahlin et al.,1994; Tripathi and Mishra, 2007). Gossypol is a yellow, polyphenolic aldehyde compound, which is present in the highest concentrations in WCS pigment glands (Blauwiekel et al., 1997). Gossypol exists in both free and bound forms in WCS. Gossypol is thought to bind proteins containing free amino sites, which impairs absorption in the digestive tract (Reiser and Fu, 1962; Barraza et al., 1991). Other deleterious effects of gossypol are embryotoxicity, growth retardation, edema of the lungs and impaired reproductive function (Arieli, 1998; Jo et al., 2003).

Reduction of ruminal CP degradation of oilseeds and removal of their anti-nutritional factors are essential to improve the nutritional quality of them and effectively utilize their potential as feed for high producing dairy cows and other animals. Several conventional processing methods have been used in order to decrease ruminal CP degradation and anti-nutritional factors of feeds, such as extrusion, roasting, toasting, Jet-Sploding and microwaving (Deacon et al., 1988; Wang et al., 1999; Fathi Nasri et al., 2008, Ebrahimi et al., 2010). The above mentioned treatments reduce ruminal CP degradation and anti-nutritional factors, but destruction of some nutrients and reduction of CP digestibility may occur with heat treatments.

Food irradiation is a process where food is exposed to ionizing radiations such as gamma rays emitted from the radioisotopes ^{60}Co and ^{137}Cs, or, high energy electrons and X-rays produced by machine sources (Farkas, 2006). The use of irradiation technology is promising since its effect on nutrients is minimal if suitable

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doi:10.1016/j.radphyschem.2011.08.003

doses are applied. EB-irradiation has been proved to be successful in decontamination, disinfestation and improvement of the overall quality of food and agricultural commodities (Palekar et al., 2004; Bhat and Sridhar, 2008). Recently, EB-irradiation was effective in reducing anti-nutritional factors and increasing CP digestibility of canola meal and sorghum grains (Taghinejad-Roudbaneh et al., 2010, Shawrang et al., 2011a). However, in the literature, no information is available concerning the effects of EB-irradiation on the nutritive value of WCS, SB and CS. Therefore, the objectives of this study were to evaluate the effects of EB-irradiation on anti-nutritional factors, chemical composition and *in vitro* CP digestibility of WCS, SB and CS and to investigate ruminal degradation of CP and DM of EB-irradiated seeds.

2. Materials and methods

2.1. Sample preparation

The Iranian cultivar of SB, Williams, that is most commonly used in Iran and the CS sample, which was grown in the Hamadan province of Iran, was obtained from Oilseed Developing and Cultivation Company (Tehran, Iran). WCS sample (Varamin variety) was obtained from Varamin Cottonseed Research Center (Tehran, Iran). The seeds were cleaned by hand to remove the foreign materials.

2.2. Irradiation treatments

Two kg of WCS, SB and CS samples was divided into four equal portions and packed in polyethylene bags (18 cm × 25 cm). Three polyethylene packages of each sample were exposed to EB-irradiation at the Yazd irradiation center, Atomic Energy Organization of Iran (TT200 Rhodotron accelerator) to various doses (15, 30 and 45 kGy) at room temperature (24 °C). All samples were irradiated in the presence of air, with 4 mA beam of 10 MeV electrons. Regarding the low thickness (approximately 1.5 cm for SB and CS samples and 2.5 cm for WCS samples) of the samples packages, single sided irradiation was used. The required doses were delivered to the samples by adjusting the conveyer speed when each of the sample batches passed under the beam. The doses delivered to packages A, B and C were measured using Cellulose Three Acetate (TCA) thin film dosimeters, which showed conformity with the relevant desired doses within 7%.

2.3. Analytical procedures

Feed samples were analyzed for DM, CP, ether extract (EE) and Ash as described by AOAC (1995). The DM content was determined in feed samples and nylon bag residues by oven drying at 55 °C for 48 h. The *N* in feed samples and residues after rumen and *in vitro* incubation was determined according to AOAC method (Method 984.13, AOAC, 1995). Ash was determined by burning duplicate 2 g samples at 600 °C for 2 h in a muffle furnace (Method 942.05, AOAC, 1995). Ether extraction was determined after extraction with ether (Method 920.39, AOAC, 1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by the method of Van Soest et al. (1991), using an automatic fiber analyzer (Fibertec System M, Tecator, Hoganas, Sweden).

2.4. Phytic acid analyze of feed samples

The extraction and estimation of phytic acid in the feed samples were done according to De Boland et al. (1975). Briefly, samples (2 g) were extracted (24 h) with a solution containing 12 ml/l HCl and 100 g/l Na_2SO_4 and centrifuged at 1430 × *g* for 15 min at room temperature. Then, 10 ml of extract was diluted with 10 ml of water and treated with 5 ml solution of 4 g/l $FeCl_3$ in 6 ml/l HCl containing 50 g/l Na_2SO_4. After boiling for 15 min, the samples were centrifuged at 3220 × *g* for 15 min at room temperature. Analysis of phosphorus content of the insoluble ferric salt was determined colorimetrically at 660 nm after digestion with 3 ml of sulfuric acid and 5 ml nitric acid. Phytic acid was calculated on the assumption that it contains 282 g/kg of phosphorus (De Boland et al., 1975).

2.5. Gossypol analyze of WCS

Free gossypol was determined according to ISO assay (ISO 6866, 1985). Briefly gossypol extraction was completed with a mixture of 2-propanol and hexane. Then gossypol was converted to gossypol-dianiline using aniline. Measurement of the absorbance was conducted at the wavelength of maximum absorbance, i.e. 440 nm.

2.6. Glucosinolate analyze of CS

Total glucosinolate content of CS samples were determined according to Clifford and Smith (1987). Briefly, glucosinolates were hydrolyzed with myrosinase to liberate glucose. Glucose generated during hydrolysis was converted to glucose 6-phosphate, which was measured spectrophotometerically.

2.7. Trypsin inhibitor activity assay of SB

The method of Kakade et al. (1969) was used to determine trypsin inhibitor activity of SB by using *N*-benzoyl-arginine-*p*-nitroanilide (BAPNA) at a concentration of 30 mg/100 ml as a substrate. Briefly, one gram of sample was extracted with 15 ml of citrate buffer (pH 4.6), stirred for 2 h at room temperature and then centrifuged at 1810 × *g* for 20 min. The reaction mixture consisted of 0.1 ml of the extract, 0.9 ml of distilled water, 7 ml of the substrate and 1 ml of trypsin enzyme at concentration of 4 mg/100 ml of 0.001 M HCl. Trypsin inhibitor activity, expressed as trypsin inhibitor units/mg sample, was calculated from the absorbance read at 410 nm.

2.8. In vitro CP digestibility of feed

Digestibility of rumen undegraded CP was estimated using the three-step *in vitro* procedure of Calsamiglia and Stern (1995). Briefly, untreated and irradiated samples (approximately 1.5 g of each feeds) were ground through a 2 mm screen and were placed into 6 cm × 10 cm bags. Bags were suspended in the rumen for 16 h. After the incubation period, bags rinsed with tap water until runoff was clear and were dried at 55 °C for 48 h. Samples of the ruminal undegradable fraction of each feeds containing 15 mg N were incubated for 1 h in 10 ml solution (0.1 N HCl) containing 1 g/l of pepsin (Sigma P-7012, Sigma Chemical, St. Louis, MO). Following incubation in a 38 °C shaker water bath, pH was neutralized with 0.5 ml of 1 N NaOH and 13.5 ml of a pancreatin (Sigma P-7545, Sigma Chemical, St. Louis, MO) solution. Samples were incubated for 24 h at 38 °C in a shaker water bath, then 3 ml of a 100% (wt/vol) solution of trichloroacetic acid (TCA) were added to stop enzymatic action and precipitate undigested proteins. Samples were centrifuged at 10,000 × *g* for 15 min, after that supernatants were analyzed for soluble N. *In vitro* digestion of protein was calculated as TCA-soluble N divided by amount of initial sample N (i.e., nylon bag residues).