Journal of Animal Sciences and Livestock Production
ISSN: 2577-0594

Open Access Research Article

Effects of Gamma Ray Irradiation and Electron Beam, Microwaving, and Roasting on the Chemical Composition, Ruminal Degradability, and *In vitro* Digestibility of Cottonseed

Roohollah Ebrahimi Mahmoodabad*

Department of Animal Science, Islamic Azad University, Tehran, Iran

ABSTRACT

This study examines the effects of gamma and electron beam irradiation, microwaving, and roasting on cottonseed's chemical composition, ruminal degradability, and *in vitro* digestibility to enhance its use as animal feed. All treatments significantly increased crude protein levels and digestibility. Electron beam irradiation, microwaving, and roasting notably reduced ruminal degradability and improved nutritional value. Electron beam irradiation was the most effective, followed by microwaving and roasting. These findings suggest that such processing methods enhance the quality and nutritional value of cottonseed.

Keywords: Irradiation; Microwaving; Roasting; Cottonseed; Degradability; Digestibility

INTRODUCTION

When high-quality protein is fed to ruminants, it is extensively degraded by the microorganisms in the rumen. Most of the protein is converted into peptides, amino acids, and ammonia. In such cases, the benefits of protein quality, including amino acid balance and digestibility, are lost. Oilseeds and their meals are valuable protein sources utilized in ruminant diets. However, their rumen degradability is high, and they also contain anti-nutritional elements that prevent their widespread use in diets.

Over the past few decades, various processing methods have been studied to improve the nutritional value of oilseeds and their by-products by reducing rumen protein degradability, eliminating anti-nutritional factors, and increasing post-rumen digestibility. These methods include chemical treatments (using alcohol, xylose, organic and mineral acids, lignosulfonate, and formaldehyde), physical treatments (using dry heat, moist heat, and irradiation), or a combination of these. Chemical processing methods have been criticized for environmental pollution and, in some cases, negatively affect animal products [1].

Therefore, physical processing methods have been more frequently employed. Among the physical methods, thermal processes, such as roasting, are the most common. Heat reduces protein degradability in the rumen due to denaturation or the formation of cross-linkages between protein-carbohydrate (Maillard reactions) and protein-protein bonds. In the study by Taghinejad-Roodbaneh et al., the roasting process significantly reduced the degradability of dry matter and crude protein in the rumen, decreased the gossypol content, and, in turn, increased the intestinal digestibility of cottonseed. Another effective and

Received:	26-September-2024	**Manuscript No:**	IPJASLP-24-21621
Editor assigned:	28-September-2024	**PreQC No:**	IPJASLP-24-21621 (PQ)
Reviewed:	11-October-2024	**QC No:**	IPJASLP-24-21621
Revised:	14-June-2025	**Manuscript No:**	IPJASLP-24-21621 (R)
Published:	21-June-2025	**DOI:**	10.36648/2577-0594.9.1.40

Corresponding author: Roohollah Ebrahimi Mahmoodabad, Department of Animal Science, Islamic Azad University, Tehran, Iran; E-mail: sayyedroohollah.ebrahimi@yahoo.com

Citation: Mahmoodabad RE (2025) Effects of Gamma Ray Irradiation and Electron beam, Microwaving, and Roasting on the Chemical Composition, Ruminal Degradability, and *In vitro* Digestibility of Cottonseed. J Anim Sci Livest Prod. 9:40.

environmentally benign physical feed processing method is irradiation [2].

It appears that irradiation offers more advantages over previously mentioned methods due to less damage to nutrients, especially proteins, no formation of indigestible products such as Maillard products, removal of microbial and fungal contaminants, no residual effects, and increased nutrient digestibility. Food irradiation involves the controlled use of ionizing radiations, including electron beam and gamma rays, to enhance food quality. Studies have shown that irradiation is effective in reducing rumen degradability and eliminating anti-nutritional elements in oilseeds and their meals. Using short waves, another physical method has recently gained attention in food processing. This method is essentially considered a form of thermal irradiation (non-thermal irradiation includes electron beam and gamma-ray exposure, which has been mentioned).

Short waves are part of the electromagnetic energy spectrum. They travel at the speed of light in a straight line. The shorter the wavelength, the more energy is transmitted by the wave. Short wavelengths lie between radio waves and visible light. Sadeghi and Shorong and Taghinejad-Roodbaneh et al. reported the effects of short-wave irradiation (800 watts) for 2, 4, and 6 minutes on reducing the degradability of crude protein and anti-nutritional elements in cottonseed and canola meals.

Cottonseed is a good source of energy, crude protein, and fiber in the diets of domestic animals. It contains 2.22 mega-calories per kilogram of net energy for lactation, 23% crude protein, 44% insoluble fiber in a neutral detergent, and 34% in an acid detergent. Typically, cottonseed makes up about 15% of the diet in ruminants. However, its high unsaturated fat content and impact on rumen fiber degradation, crude protein degradation, and high content of the anti-nutritional substance gossypol limit its use in diets to 15%. Appropriate processing methods can increase the level of its usage in diets. This research examined the effects of ionizing radiations, including gamma ray and electron beam, microwaves, and roasting, on cottonseed's chemical composition, rumen degradability, and *in vitro* digestibility.

MATERIALS AND METHODS

This research was conducted at the dairy unit and the Central Laboratory of the National Institute of Animal Science in Karaj, Iran. Cottonseed samples were obtained from the development company of oil seed cultivation. These samples were then prepared for processing. Gamma-ray irradiation of the cottonseed was conducted at the Radiation Application Research Institute, Atomic Energy Organization of Iran. Prior to irradiation, the moisture content of the samples was adjusted to 25% and then irradiated at room temperature with doses of 20 and 40 kilogray. The irradiation was performed using a laboratory system, model PX-30, at an average rate of 0.34 gray per second. In this system, Cobalt-60 was used as the gamma radiation source. To control the quality of irradiation, or more precisely, to determine the relationship between irradiation time and the absorbed dose by the material, the irradiation chamber was calibrated using a reference chemical dosimeter system based on the ASTM E1026-95 standard.

The electron beam was performed at the Yazd Radiation Processing Center, Atomic Energy Organization of Iran. For this purpose, the Rhodotron electron accelerator (Model TT-2200, IBA Co., Belgium) was used. The cottonseed samples were irradiated with an electron beam of 10 mega electron-volts and an electron beam current of 6 milliamperes at doses of 25, 50, and 75 kilogray, with an error of less than 5%. The irradiation method was unidirectional. The samples were exposed to the electron beams multiple times to achieve the required doses. The dose measurement was carried out using a polystyrene calorimeter (reference dosimeter) to ensure accuracy in the administered doses. Microwave processing was conducted in a microwave oven at 800 watts for 3 and 5 minutes, and the roasting process was carried out in an oven at 140°C for 15 and 30 minutes.

Chemical Composition

The chemical composition of the cottonseed samples was determined, including dry matter, ash, crude protein, and ether extract according to standard AOAC methods, the insoluble fiber in a neutral detergent, and insoluble fiber in acid detergent according to the method of van Soest et al., and acid detergent insoluble nitrogen according to the method of Licitra et al.

Experimental Animals and Diets

Three Dashtyari breed male cows (average weight ± 10 of 2970 kg), each equipped with a ruminal fistula, were used to perform digestibility and degradability experiments. The animals were housed in cages (3 m × 2 m). Their diet was formulated according to the standards required for maintenance (**Table 1**). Feeding was done twice daily, in the morning (at 8 AM) and afternoon (at 4 PM). Additionally, the animals had free access to drinking water.

Degradability Experiment

The degradability of the samples' dry matter and crude protein was determined using the nylon bag technique. Initially, the cottonseed samples were ground through a 2 mm sieve. Then, 6 grams of each sample were incubated in the rumen of the cows through the fistula at times 0, 2, 4, 6, 12, 16, 24, and 48 hours. After incubation, the nylon bags containing the residues were removed from the rumen and immediately washed in a washing machine with cold water. After drying in an oven at 55°C for 48 hours, the weight difference of the bag contents before and after incubation was considered the amount of dry matter disappearance. The crude protein content of the residues inside the bags was also measured to determine the degradability of this nutrient and its related parameters. Various parameters of dry matter and crude protein degradability were calculated using the following equations:

$$P = a + b(1 - e^{-ct}) \quad (1)$$

$$ERD = a + \left[\frac{b \times c}{c \times k}\right] \quad (2)$$

P is the potential degradability or disappearance at time t,

ERD is the effective rumen degradability percentage,

a is the quickly degradable fraction,

b is the slowly degradable fraction,

c is the rate constant of degradation,

k is the rate constant of passage of digesta from the rumen,

t is the residence time of the sample in the rumen (hours),

e is the base of natural logarithms (approximately 2.718).

Protein Digestibility

A two-stage digestion method was employed to assess the digestibility of cottonseed samples, based on Tilley and Terry's methodology (1963). Initially, the samples were ground to pass through a 1 mm sieve and subsequently dried in an oven [3]. Each sample, weighing 0.5 grams, was placed into 100 mL Erlenmeyer flasks, with three replicates per sample and three additional Erlenmeyer flasks serving as untreated controls. Rumen fluid was collected before morning feeding *via* a ruminal fistula and filtered through two layers of unique fabric for laboratory use.

After introducing carbon dioxide, the Erlenmeyer flasks were sealed and placed in a warm water bath at 39°C.

Table 1: Feed ingredients and chemical composition of experimental diets.

Feed ingredients	Percentage of dry matter (%)
Alfalfa	50
Wheat straw	16.67
Barley	26.67
Corn grain	3.33
Wheat bran	1.66
Cottonseed meal	1.33
Vitamin and mineral supplement	0.34
Chemical composition of the diet	**Percentage of dry matter (%)**
Dry matter	88.88
Crude protein	13.04
Neutral Detergent Fiber (NDF)	37.29
Acid Detergent Fiber (ADF)	34.99
Calcium	0.74
Phosphorus	0.26
Metabolizable energy (MJ/kg of dry matter)	2.27

Artificial saliva was prepared by dissolving 8.9 grams of sodium bicarbonate, 3.71 grams of disodium hydrogen phosphate dihydrate, 0.57 grams of potassium chloride, 0.47 grams of sodium chloride, and 0.12 grams of magnesium sulfate in double-distilled water, bringing the total volume to 1 liter in a volumetric Erlenmeyer flask. Forty milliliters of this solution were added to each sample Erlenmeyer flask. Approximately 30 minutes before initiating anaerobic digestion, 1 mL of 4% (w/v) calcium chloride per liter of artificial saliva was introduced, and the solution was carbonated for 10 to 15 minutes to adjust the pH to between 6.9 and 7.0. Warmed in the water bath, the prepared artificial saliva and rumen fluid were mixed at 4:1 and bubbled with carbon dioxide for approximately 4 to 5 minutes.

Each control and sample-containing Erlenmeyer flask received 50 mL of the mixture and was immediately sealed after introducing carbon dioxide for 15 seconds. The Erlenmeyer flasks remained in the 39°C water bath for 48 hours, shaken regularly at set intervals. After the anaerobic digestion phase, the Erlenmeyer flasks were removed, and their caps were gently opened. The digestibility process then continued by adding 6 mL of 20% hydrochloric acid in three stages to each

Erlenmeyer flask, followed by 2 mL of 20% pepsin solution. The Erlenmeyer flasks were resealed and returned to the 39°C water bath for an additional 46 hours, during which they were periodically shaken, similar to the initial digestion stage.

After the pepsin digestion phase was complete, the contents of each Erlenmeyer flask were filtered through Whatman No. 41 filter paper using a Büchner funnel and a vacuum pump. The undigested residues were gently separated from the Büchner funnel, folded, and dried in an oven at 105°C for 24 hours. The dry weight of these residues was then determined. The filter papers were subsequently incinerated in an electric furnace at 560°C for 4 hours to determine the amount of crude ash in the undigested residues.

Statistical Analysis

Variance analysis for chemical composition and *in vitro* digestibility: The data analysis derived from the chemical composition and *in vitro* ruminal digestibility was performed using a Completely Randomized Design (CRD). The Analysis of Variance (ANOVA) was applied according to the following model:

$$Y_{ij}=\mu+T_i+e_{ij} \quad (3)$$

where Y_{ij} represents the value of each observation, μ is the overall mean, T_i is the treatment effect (processing method), and e_{ij} denotes the experimental error.

Variance analysis for digestibility testing: For the digestibility testing, data analysis was carried out using a Randomized Complete Block Design (RCBD), and the variance was analyzed based on this model:

$$Y_{ij}=\mu+T_i+B_j+e_{ij} \quad (4)$$

In this model, Y_{ij} denotes the observation value, μ is the overall mean, T_i indicates the treatment effect, B_j is the block effect (animal), and e_{ij} represents the experimental error. Data processing was executed using SAS statistical software, employing the ANOVA procedure. The least significant difference test was utilized to compare means.

RESULTS AND DISCUSSION

Chemical Composition

The effects of various processing methods on the chemical composition of cottonseed are presented in Table 2. There were significant differences between the treatments ($p<0.05$). The gamma ray and electron beam treatments observed the lowest dry matter content, averaging 80.80%. Microwave irradiation for 3 and 5 minutes and roasting for 15 and 30 minutes did not affect the dry matter percentage. The crude protein content of untreated cottonseed (control) was 19.02% dry matter. All treatments increased the crude protein content, with the highest amounts observed in samples treated by roasting for 15 and 30 minutes, with values of 22.84% and 22.74%, respectively. No specific reason for the decrease in dry matter percentage under gamma or electron beam irradiation treatments was mentioned in the available reports, but it seems that changes in the percentage of components, such as insoluble fibers in neutral and acid detergent, might contribute to these changes [4].

Among the different treatments, the highest crude ash content was observed in samples treated with microwave for 5 minutes, amounting to 5.70%. This value was 4.25% for control samples and averaged 3.87% for other treatments. The increase in ash content under microwave treatment for 5 minutes, while similar treatments had lower levels, could relate to external factors affecting the measurement process, specific sample characteristics, or experimental errors in determining the ash percentage.

The lowest neutral detergent fiber was found in samples treated with microwave for 3 and 5 minutes (36.17% and 30.07% dry matter, respectively), and the lowest acid detergent fiber was in gamma irradiation at 40 kGy (36.75%), electron beam at 20 kGy (37.20%), and microwave for 3 minutes (36.09%). The 40 kGy electron beam reduced the insoluble protein in acid detergent compared to the control (3.05% dry matter *vs.* 3.44% dry matter). Other treatments did not differ significantly from the control or were higher. Salari et al. reported that electron beam irradiation did not significantly affect crude protein, ether extract, and ash, but a 40 kGy dose decreased the crude fiber content.

In reports by Nayfi et al., gamma-ray irradiation and electron beam did not significantly affect the organic matter, ether extract, and crude protein of cottonseed, but increased doses of electron beam reduced the crude fiber content. In the study by Bahreini et al., gamma–ray irradiation and electron beam cottonseed have reduced the lignin content, which aligns with the current research. Taghinejad and colleagues reported that roasting (15 and 30 minutes) did not affect ash, crude protein, or the cell wall components of cottonseed. In other studies, gamma irradiation (15, 30, and 45 kGy) and microwave (2, 4, and 6 minutes at 800 watts) did not affect the chemical composition of cottonseed and Mendab seed. Shahbazi et al. showed that the electron beam decreased the insoluble fibers in neutral and acid detergents of wheat and barley straw [5]. One of the primary goals of processing feed resources is to reduce the content of insoluble fibers in neutral and acid detergents, which was confirmed in this experiment. In this experiment, the above results were confirmed.

Alberti et al. explained that ionizing radiations, by generating ions and free radicals, cause depolymerization of complex compounds, especially separating bonds between cellulose and other compounds and breaking covalent bonds. Therefore, the reduction in insoluble fiber parts under gamma ray and electron beam is the dissolution of cellulose and, to a greater extent, the release of hemicellulose (as it is freed from cellulose). Shorong et al. attributed the decrease in crude fiber due to irradiation to the oxidation of cellulose and the conversion of cellulose and lignin into soluble components. Ta'han et al. stated that the reduction in cell wall components due to irradiation is due to lignin removal, polymer degradation, and the destruction of the crystalline structure of cellulose.

Table 2: Effect of different processing methods on chemical composition of cottonseed.

Treatment	DM[1] (%)	CP[2] (%)	NDF[3] (%)	ADF[4] (%)	ADICP[5] (%)	Ash (%)
Unprocessed	86.13^{a}	19.02^{e}	55.24^{a}	44.85^{a}	3.44^{d}	4.25^{b}
Gamma irradiation 20 kGy	81.06^{c}	21.40^{cd}	46.05^{c}	41.15^{c}	3.63^{c}	4.00^{cb}
Gamma irradiation 40 kGy	80.78^{c}	22.17^{b}	50.65^{b}	36.75^{d}	3.48^{cd}	3.84^{c}
Electron beam 20 kGy	80.88^{c}	22.67^{ab}	42.97^{d}	37.20^{d}	3.63^{c}	4.00^{bc}
Electron beam 40 kGy	80.49^{c}	21.85^{c}	41.50^{e}	42.81^{b}	3.05^{e}	3.79^{c}
Microwave 3 minutes	84.15^{ab}	20.87^{c}	36.17^{g}	36.09^{d}	3.91^{b}	3.85^{c}
Microwave 5 minutes	86.82^{a}	21.65^{cd}	30.07^{h}	40.35^{c}	5.77^{a}	5.70^{a}
Roasting 15 minutes	87.25a	22.84^{a}	38.85^{f}	39.89^{c}	3.38^{d}	3.92^{bc}
Roasting 30 minutes	86.87^{a}	22.74^{a}	37.97^{f}	40.72^{c}	3.34^{d}	3.69^{c}
SEM[6]	0.48	0.42	0.45	0.39	0.05	0.09
Significance level	0.001	0.001	0.001	0.001	0.001	0.001

Note: Values with different letters in each column are significantly different (P<0.05). 1: Dry matter, 2: Crude protein, 3: Neutral detergent fiber, 4: Acid detergent fiber, 5: Acid detergent insoluble crude protein, 6: Standard error of means

Effective Rumen Degradability of Dry Matter

Various parameters of degradability and effective rumen degradability of dry matter for untreated and differently treated cottonseed samples are displayed in Table 3. For untreated samples (control), the quickly and slowly degradable fractions were 19.30% and 51.90%, respectively. Treatment processes reduced the quickly degradable fraction, with the lowest value (11.70%) observed in samples irradiated with a 40 kGy electron beam. However, this difference was not statistically significant compared to samples irradiated with gamma rays at doses of 20 and 40 kGy (12.70% and 12.20%, respectively), electron beam at 20 kGy (13.10%), or samples roasted for 30 minutes (13.90%).

Except for the electron beam treatment at 20 kGy and microwave treatment for 3 minutes (51.00% and 51.40%, respectively), other treatments increased the slowly degradable fraction significantly (p<0.05). The highest amounts were in samples roasted for 15 and 30 minutes and microwaved for 5 minutes (60.60%, 62.90%, and 62.00%, respectively). Microwave (5 minutes) and roasting processes (15 and 30 minutes) increased the degradability potential compared to the control (p<0.05) (76.90%, 75.70%, and 76.70%, respectively) [6]. Other treatments did not show significant differences from the control. All treatments uniformly reduced the rate constant of degradation.

The effective dry matter degradability for untreated cottonseed at passage rates of 2%, 5%, and 8% per hour were 64.82%, 56.30%, and 50.80%, respectively. At passage rates of 2% and 5% per hour, the dry matter degradability of samples treated with ionizing rays (gamma ray and electron beam at doses of 20 and 40 kGy) and microwave for 3 minutes was significantly reduced (p<0.05) (respectively 57.10%, 57.80%, 55.10%, 55.90% for 2% passage rate and 46.90%, 47.30%, 45.90%, 48.10%, 46.50% for 5% passage rate). At an 8% passage rate, all treatments significantly reduced dry matter's effective degradability (p<0.05). In this regard, treatments with gamma ray and electron beam at 20 and 40 kGy doses and microwave for 3 minutes showed a more pronounced average reduction of 20% ineffective degradability among the treatments. Treatments with microwave for 5 minutes and roasting for 15 and 30 minutes are followed by an average reduction of 10%.

Ha'hum et al. demonstrated that a 10 kGy gamma-ray dose reduced the dry matter degradability of cottonseed. Taghinejad et al. reported that roasting for 30 minutes and microwaving for 6 minutes reduced the quickly and slowly degradable fractions of cottonseed. Additionally, Rezaeian et al. demonstrated that gamma ray and electron beam irradiation at doses of 20 and 40 kGy, as well as roasting for 15 minutes, significantly reduced the effective degradability of dry matter and crude protein in soybean seeds at passage rates of 2%, 5%, and 8% per hour.

Akbarian et al. reported that irradiating soybeans with a 63 kGy electron beam dose increased the quickly degradable fraction, reduced the slowly degradable fraction, and increased the effective degradability of dry matter. Sadeghi et al. observed that microwaving soybean meal decreased the quickly degradable fraction and increased the slowly degradable fraction, and the degradation rate also showed a decreasing trend. Researchers have reported that gamma irradiation at various levels causes a reduction in the quickly degradable fraction and an increase in the slowly degradable fraction of dry matter and crude protein, which indicates that the protein and nutrients are being protected by gamma irradiation processing. Nobar reported that microwaving soybeans with a power of 900 watts for 3, 4, and 5 minutes increased the slowly degradable fraction and reduced the quickly degradable fraction, constant degradation rate, and effective degradability of dry matter at rumen passage rates of 0.02%, 0.05%, and 0.08% per hour of dry matter [7]. The reduction in dry matter degradability could be due to the formation of complexes between proteinaceous and non-proteinaceous compounds, such as starch, reducing the accessibility of rumen microorganisms to them. Irradiation by gelatinizing starch and protein and limiting their degradability could reduce the degradability of dry matter.

Table 3: Effect of different processing methods on effective ruminal dry matter degradability of cottonseed.

Treatment	Effective dry matter degradability at passage rate (per hour)					
	0.02	0.05	0.08	a	b	a+b
Unprocessed	64.82^{a}	56.30^{a}	50.80^{a}	19.30^{a}	51.90^{d}	71.20^{b}
Gamma irradiation 20 kGy	57.10^{bc}	46.90^{b}	40.50^{c}	12.70^{bcd}	55.50^{c}	68.20^{cd}
Gamma irradiation 40 kGy	57.80^{bc}	47.30^{b}	40.70^{c}	12.20^{cd}	57.00^{b}c	64.80^{bc}
Electron beam 20 kGy	55.10^{c}	45.90^{b}	40.00^{c}	13.10^{bcd}	51.00^{d}	64.50^{c}
Electron beam 40 kGy	58.80^{b}	48.10^{b}	41.40^{c}	11.70^{d}	58.60^{b}	70.30^{bc}
Microwave 3 minutes	55.90^{b}c	46.50^{b}	40.60^{c}	14.60^{b}	51.40^{d}	66.00^{bc}
Microwave 5 minutes	64.20^{a}	53.00^{a}	46.00^{b}	14.10^{bc}	62.00^{a}	76.90^{a}
Roasting 15 minutes	63.70^{a}	52.50^{a}	45.50^{b}	14.70b	60.60^{a}	75.70^{a}
Roasting 30 minutes	64.40^{a}	53.00^{a}	45.80^{b}	13.90^{bcd}	62.90^{a}	76.70^{a}
SEM^{2}	1.02	1.26	1.32	0.69	0.77	0.82
Significance level	0.001	0.001	0.002	0.001	0.001	0.001

Note: Values with different letters in each column are significantly different (P<0.05). 1-a: Washout (soluble) fraction, b: Potentially degradable fraction, c: Degradation rate of b fraction.

2-SEM: Standard Errors of Means.

Effective Rumen Degradability of Protein

Various parameters of degradability and effective rumen degradability of crude protein in untreated and treated cottonseed samples are presented in **Table 4**. For untreated samples (control), the washout (soluble) fraction, potentially degradable fraction, and degradation rate of the 'b' fraction were 27.30%, 61.00%, and 0.019/hour, respectively. All treatments reduced the washout fraction significantly (p<0.05). The lowest values were observed in samples microwaved for 5 minutes and roasted for 30 minutes, which were 12.80% and 13.00%.

The potentially degradable fraction was increased by gamma irradiation at doses of 20 and 40 kGy (71.30% and 71.60%), microwaving for 5 minutes (68.10%), and roasting processes for 15 and 30 minutes (65.10% and 66.50%). Electron beam (20 and 40 kGy) and microwaving for 3 minutes had no significant effect on this trait (64.10%, 53.70%, and 62.90%, respectively). Other treatments reduced the degradation rate constant except for the 20 kGy gamma irradiation dose (0.018/hour). The lowest value was observed in the roasting process for 30 minutes (0.007/hour). The effective degradability of crude protein in untreated cottonseed at passage rates of 2%, 5%, and 8% per hour were 82.06%, 75.07%, and 70.40%, respectively. At passage rates of 2% and 5% per hour, except for the gamma irradiation doses of 20 and 40 kGy (respectively 84.40% and 81.30% for 2% passage rate, and 75.90% and 71.20% for 5% passage rate), and at the 8% passage rate except for the 20 kGy gamma irradiation dose (65.50%), all treatments reduced the effective degradability of crude protein. The lowest values were observed in samples roasted for 30 minutes (respectively 64.90% and 44.30% for 2%, 5%, and 8% passage rates).

Taghinejad et al. showed that a 15 kGy gamma irradiation dose had no effect on the rumen degradability of crude protein in cottonseed, but as the irradiation dose increased to 30 and 45 kGy, the degradability decreased. Ha'hum et al. reported that gamma irradiation at a 10 kGy dose reduced the degradability of crude protein in cottonseed. Existing studies indicate that microwave irradiation reduces the protein degradability of cottonseed and canola meal. Ghanbari et al. demonstrated that gamma irradiation decreased the protein degradability in cottonseed. Additionally, Rezaeian et al. demonstrated that electron beam irradiation at doses of 20 and 40 kGy and microwave processing for 3 and 5 minutes reduced the crude protein degradability of soybean seeds.

Table 4: Effect of different processing methods on effective ruminal crude protein degradability of cottonseed.

Treatment	Effective crude protein degradability (Percentage) at passage rate (per hour)			Degradability parameters[1]			
	0.02	0.05	0.08	a	b	a+b	c
Unprocessed	82.6[a]	75.7[a]	70.4[a]	27.3[a]	61.0[d]	88.3[a]	0.019[a]
Gamma irradiation 20 kGy	84.4[a]	75.9[a]	69.5[a]	20.3[b]	71.3[a]	91.6[a]	0.018[a]
Gamma irradiation 40 kGy	81.3[a]	71.2[a]	64.3[b]	19.0[bc]	71.6[a]	90.6[a]	0.014[b]
Electron beam 20 kGy	72.1[b]	62.1[b]	55.2[c]	17.8[bcd]	64.1[bcd]	81.8[b]	0.011[bc]
Electron beam 40 kGy	71.5[b]	62.4[b]	55.9[c]	16.1[abc]	53.7[bcd]	79.7[b]	0.013[bc]
Microwave 3 minutes	69.3[bc]	58.8[b]	51.8[c]	16.9[bcd]	62.9[cd]	79.8[b]	0.009[d]
Microwave 5 minutes	70.5[bc]	59.7[b]	52.3[c]	12.8[c]	68.1[ab]	80.9[b]	0.011[cd]
Roasting 15 minutes	71.8[b]	62.0[b]	55.2[c]	14.9[dc]	65.1[bc]	80.9[b]	0.013[bc]
Roasting 30 minutes	64.9[c]	52.1[c]	44.3[d]	13.0[c]	66.5[bc]	79.5[b]	0.007[c]
SEM[2]	1.54	0.01	1.47	1.2	1.45	1.64	0.001
Significance level	0.001	0.001	0.001	0.001	0.008	0.001	0.001

Note: Values with different letters in each column are significantly different (P<0.05). 1-a: Washout (soluble) fraction, b: Potentially degradable fraction, c: Degradation rate of b fraction. 2-SEM: Standard Errors of Means.

Digestibility

The effects of different processing methods on the *in vitro* digestibility of cottonseed are presented in Table 5. The digestibility of dry matter, organic matter, and organic matter in dry matter for the control samples were 30.93%, 28.26%, and 27.17%, respectively. All treatments significantly increased these values (p<0.05). Among the various treatments, the roasting process for 30 minutes had the most significant effect, improving the figures to 44.00%, 41.70%, and 40.73%, respectively. The digestibility of organic matter in samples processed with 40 kGy gamma irradiation (40.64%) and the digestibility of organic matter in dry matter in samples irradiated with 40 kGy electron beam (37.90%) showed no significant difference compared to those roasted for 30 minutes [8]. In the study by Taghinejad Roodbaneh et al., roasting for 5 and 30 minutes, gamma irradiation at doses of 30 and 45 kGy, and microwave irradiation for 6 minutes all increased the intestinal digestibility of cottonseed protein, which is consistent with the results of the current research. Ha'hum et al. reported that gamma irradiation increased the digestibility of cottonseed. Ghanbari et al. found that gamma ray and electron beam at doses of 25, 50, and 75 kGy did not significantly affect the digestibility of cottonseed.

Irradiation reduces damage to nutrients, including proteins, does not create indigestible products like Maillard products, removes microbial and fungal contaminants from feed materials, eliminates anti-nutritional factors, and increases the digestibility of nutrients. Like thermal processes, irradiation causes protein denaturation and exposes hydrophobic amino acids. The hydrophobicity leads to aggregation and subsequent coagulation and precipitation, reducing degradability but increasing intestinal digestibility. Irradiation enhances the enzymatic digestibility of the feed and ultimately improves overall gastrointestinal digestibility. Shorong et al. attributed the increased digestibility of meals due to irradiation to the breakdown of hydrogen bonds and other weak non-covalent bonds, changes in amino acid positioning, and increased protein surface hydrophobicity. Ionizing radiations like gamma ray and electron beam and thermal processing such as roasting cause denaturation and expose hydrophobic amino acids, which reduce protein solubility and, hence, rumen degradability. However, this "bypass" fraction is highly digestible in the intestine because hydrophobic amino acids are more susceptible to intestinal proteases such as trypsin, chymotrypsin, and carboxypeptidase. This way, the intestinal digestibility of irradiated protein is enhanced. Additionally, ionizing radiations have a high capability to eliminate anti-nutritional elements (even at low doses, significant amounts of anti-nutritional substances are removed) such as gossypol found in cottonseed, which increases the intestinal digestibility of protein.

Table 5: Effect of different processing methods on *in situ* digestibility of cottonseed.

Treatment	DDM[1] (%)	DOM[2] (%)	DOMD[3] (%)
Unprocessed	30.93^{f}	28.26^{f}	27.17^{e}
Gamma irradiation 20 kGy	36.38^{d}	34.66^{d}	32.91^{cd}
Gamma irradiation 40 kGy	41.30^{b}	40.64^{a}	35.34^{bcd}
Electron beam 20 kGy	38.39^{c}	34.80^{d}	32.98^{cd}
Electron beam 40 kGy	41.05^{b}	38.87^{b}	37.90^{ab}
Microwave 3 minutes	37.75^{c}	35.60^{d}	33.29^{cd}
Microwave 5 minutes	35.13^{e}	32.61^{e}	31.97^{d}
Roasting 15 minutes	40.25^{b}	37.25^{c}	36.15^{bc}
Roasting 30 minutes	44.00^{a}	41.70^{a}	40.73^{a}
SEM[2]	0.38	0.43	1.14
Significance level	0.001	0.001	0.007

Note: Values with different letters in each column are significantly different (P<0.05). 1-DDM: Dry matter digestibility; 2-DOM: Organic matter digestibility; 3-DOMD: Organic matter digestibility in dry matter, 2-SEM: Standard Errors of Means.

Rumen Degradability of Cottonseed Amino Acids

This study examined the effects of various processing methods on reducing essential and non-essential amino acids in cottonseed before ruminal incubation (Table 6). Significant impacts (P<0.05) were noted across all processing methods. Specifically, treatments involving electron beam, microwave, and roasting resulted in a substantial reduction in both essential and non-essential amino acids in cottonseed compared to the control group (P<0.05). The gamma-ray irradiation method notably decreased the levels of lysine, methionine, isoleucine, and threonine among the essential amino acids (P<0.05), while histidine, valine, and phenylalanine did not show significant reductions compared to the control. Histidine and valine, in particular, were resistant to reduction across most processing methods.

Table 6: Effect of different processing methods on amino acids of cottonseed before incubation.

Parameter	Untreated	Gamma irradiation 20 kGy	Gamma irradiation 40 kGy	Electron beam 20 kGy	Electron beam 40 kGy	Microwave 3 minutes	Microwave 5 minutes	Roasting for 15 minutes	Roasting for 30 minutes	SEM	The significance level
	Experimental treatments										
Essential amino acids											
Histidine	^{a}0.31	^{a}0.27	^{a}0.25	^{b}0.15	^{b}0.15	^{b}0.13	^{a}0.15	^{a}0.21	^{b}0.13	0.031	<00001
Isoleucine	^{a}0.56	^{b}0.36	^{b}0.33	^{bc}0.29	^{c}0.21	^{bc}0.23	^{c}0.18	^{bc}0.25	^{c}0.19	0.037	<00001
Leucine	^{a}0.89	^{b}0.69	^{b}0.68	^{b}0.60	^{b}0.64	^{c}0.47	^{c}0.42	^{c}0.47	^{c}0.43	0.035	<00001
Lysine	^{a}0.77	^{b}0.52	^{b}0.49	^{c}0.36	^{c}0.33	^{bc}0.46	^{bc}0.46	^{c}0.36	^{c}0.32	0.038	<00001
Methionine ine	^{a}0.75	^{c}0.55	^{c}0.49	^{b}0.66	^{c}0.57	^{b}0.65	^{b}0.66	^{d}0.39	^{d}0.34	0.037	<00001
Tryptophan	^{a}0.45	^{b}0.35	^{b}0.32	^{b}0.32	^{b}0.30	^{c}0.28	^{c}0.26	^{b}0.35	^{c}0.28	0.008	<00001
Threonine	^{a}0.75	^{bc}0.47	^{bc}0.42	^{c}0.38	^{c}0.33	^{b}0.58	^{b}0.55	^{c}0.36	^{d}0.29	0.034	<00001
Valine	a0.53	^{ab}0.48	^{ab}0.45	^{ab}0.47	^{b}0.42	^{ab}0.46	b^{c}0.38	^{b}0.42	^{b}0.40	0.041	<00001
Phenyl-alanine	^{a}0.49	^{a}0.45	^{a}0.42	^{b}0.23	^{b}0.21	^{b}0.24	^{bc}0.21	^{b}0.26	^{c}0.25	0.036	<00001
Non-essential amino acids											
Serine	^{a}1.53	^{b}1.15	^{b}1.11	^{b}1.14	^{b}1.11	^{b}1.16	^{b}1.14	^{b}1.15	^{b}1.10	0.065	<00001
Glycine	^{a}0.47	^{b}0.36	^{bc}0.29	^{b}0.32	^{bc}0.29	^{b}0.36	^{b}0.33	^{bc}0.30	^{c}0.25	0.041	<00001
Arginine	^{a}2.21	^{ab}1.93	^{ab}1.90	^{b}1.56	^{c}1.38	^{b}1.73	^{c}1.35	^{b}1.77	^{c}1.39	0.093	<00001
Alanine	^{a}2.30	^{bc}1.26	^{c}1.17	^{bc}1.25	^{c}1.17	^{b}1.50	^{bc}1.28	^{c}1.11	^{c}1.15	0.068	<00001
Tyrosine	^{a}0.76	^{ab}0.67	^{ab}0.63	^{ab}0.66	^{ab}0.64	^{c}0.32	^{c}0.29	^{ab}0.62	^{b}0.59	0.048	<00001
Asparagine	^{a}2.56	^{b}1.63	^{bc}1.34	^{c}1.20	^{c}1.13	^{bc}1.33	^{c}1.18	^{c}1.19	^{cd}1.09	0.052	<00001
Aspartic acid	^{a}1.79	^{ab}1.34	^{a}1.21	^{b}1.24	^{b}1.25	^{b}1.26	^{b}1.22	^{bc}1.18	^{c}1.08	0.051	<00001
Glutamic acid+ Glutamine	^{a}0.89	^{ab}0.74	^{b}0.68	^{c}0.42	^{c}0.36	^{bc}0.49	^{c}0.39	^{bc}0.48	^{c}0.43	0.051	<00001

Note: *The averages of each row with non-common letters have a significant difference.
SEM: Standard Errors of Means

A similar trend was observed for non-essential amino acids, with microwave and roasting methods having a more pronounced effect on reducing essential and non-essential amino acids before ruminal incubation than other methods. On average, the reduction of non-essential amino acids in cottonseed samples was less significant than that of essential amino acids. Feeding livestock with protein that has low ruminal degradability can enhance animal production by allowing more amino acids to bypass the rumen and be absorbed in the intestines. The percentage of amino acids in cottonseed subjected to different treatments after 16 hours of ruminal incubation (**Table 7**) was significantly affected (P<0.05). Electron beam, microwave, and roasting treatments, with slight variations, reduced the ruminal degradability of amino acids compared to gamma-ray irradiation (P<0.05).

Table 7: Effect of different processing methods on amino acids of cottonseed after incubation.

	Experimental treatments 1										
Parameter	Untreated	Gamma irradiation 20 kGy	Gamma irradiation 40 kGy	Electron beam 20 kGy	Electron beam 40 kGy	Microwave 3 minutes	Microwave 5 minutes	Roasting for 15 minutes	Roasting for 30 minutes	SEM	The significance level
Essential amino acids											
Histidine	^{a}0.13	^{a}0.10	^{ab}0.08	^{ab}0.08	^{c}0.03	^{ab}0.09	^{c}0.04	^{ab}0.08	^{c}0.04	0.021	<0001
Isoleucine	^{a}0.11	^{a}0.09	^{ab}0.08	^{ab}0.07	^{c}0.03	^{ab}0.08	^{b}0.05	^{b}0.06	^{c}0.03	0.037	<0001
Leucine	^{a}0.17	^{ab}0.13	^{b}0.09	^{b}0.10	^{c}0.03	^{a}0.16	^{ab}0.14	^{b}0.09	^{c}0.04	0.051	<0001
Lysine	^{a}0.19	^{a}0.19	^{ab}0.13	^{b}0.11	^{c}0.08	^{ab}0.16	^{c}0.10	^{b}0.11	^{c}0.08	0.038	<0001
Methionine	^{a}0.23	^{b}0.15	^{b}0.14	^{ab}0.18	^{c}0.09	^{b}0.16	^{b}0.14	^{ab}0.19	^{c}0.11	0.037	<0001
Tryptophan	^{a}0.06	^{b}0.02	^{b}0.02	^{b}0.01	^{b}0.02	^{ab}0.04	^{b}0.02	^{a}0.06	^{ab}0.03	0.008	<0001
Threonine	^{a}0.39	^{b}0.25	^{b}0.21	^{b}0.24	^{c}0.16	^{b}0.27	^{bc}0.19	^{b}0.23	^{c}0.14	0.034	<0001
Valine	^{a}0.18	^{b}0.15	^{b}0.11	^{b}0.11	^{c}0.07	^{ab}0.16	^{c}0.09	^{b}0.11	^{cd}0.05	0.041	<0001
Phenyl-alanine	^{a}0.30	^{bc}0.16	^{b}0.19	^{a}0.27	^{c}0.13	^{b}0.19	^{c}0.13	^{b}0.21	^{bc}0.15	0.036	<0001
Non-essential amino acids											
Serine	^{a}0.55	^{b}0.44	^{b}0.39	^{c}0.21	^{c}0.25	^{b}0.45	^{b}0.39	^{b}0.48	^{b}0.44	0.071	<0001
Glycine	^{a}0.56	^{ab}0.46	^{b}0.40	^{c}0.30	^{ab}0.47	^{c}0.35	^{d}0.28	^{b}0.41	^{bc}0.37	0.043	<0001
Arginine	^{a}0.99	^{b}0.84	^{c}0.75	^{e}0.33	^{d}0.53	^{e}0.36	^{f}0.21	^{f}0.21	^{g}0.13	0.056	<0001
Alanine	^{a}2.16	^{b}1.66	^{b}1.61	^{ab}1.96	^{ab}1.82	^{bc}1.52	^{c}1.46	^{d}0.78	^{e}0.51	0.079	<0001
Tyrosine	^{a}0.75	^{b}0.63	^{c}0.55	^{d}0.47	^{d}0.47	^{e}0.27	^{f}0.21	^{e}0.26	^{g}0.17	0.047	<0001
Asparagine ine	^{a}0.27	^{ab}0.21	^{b}0.18	^{b}0.15	^{bc}0.12	^{bc}0.15	^{c}0.05	^{ab}0.25	b^{c}0.16	0.021	<0001
Aspartic acid	^{a}0.68	^{b}0.51	^{c}0.44	^{d}0.31	^{d}0.29	^{c}0.40	^{d}0.27	^{b}0.55	^{c}0.44	0.036	<0001

Glutamic acid+ Glutamine	^{a}0.88	^{b}0.77	^{bc}0.68	^{ab}0.80	^{b}0.78	^{b}0.75	^{c}0.62	^{c}0.65	^{d}0.41	0.051	<0001

Note: *The means of each row with non-common letters have a significant difference.
SEM: Standard Errors of Means

The average reduction in ruminal degradability of the essential amino acids leucine (57%), isoleucine (55%), lysine (63%), and histidine (49%) was significantly greater compared to other essential amino acids in cottonseed (P<0.05). Among these, lysine exhibited the highest reduction in ruminal degradability, averaging 42.15% in treatments involving 40 kGy electron beam irradiation and 30-minute roasting.

For non-essential amino acids, the highest average reduction in ruminal degradability was observed in arginine (42.02%), tyrosine (50.50%), and asparagine (58.79%). In this context, processing methods such as electron beam, microwave, and roasting proved more effective in reducing the ruminal degradability of non-essential amino acids than gamma-ray irradiation. The reduction trend was particularly noticeable for serine with electron beam irradiation and glycine and aspartic acid using both electron beam and microwave methods. Additionally, roasting effectively reduced the ruminal degradability of arginine, alanine, tyrosine, glutamic acid, and glutamine [9].

The findings of this study indicate that untreated cottonseed amino acids exhibit high ruminal degradability. Various processing methods effectively reduced this degradability, thereby increasing intestinal digestibility and altering cottonseed's absorption site of crude protein and amino acids. Moderate thermal processing and irradiation with acceptable doses of electron beam and gamma rays are expected to reduce ruminal degradability and enhance the intestinal availability of amino acids.

A significant challenge in optimizing this process lies in the variability of heat and irradiation treatment results, largely due to inconsistent management of processing factors. Achieving optimal temperature, effective irradiation dose, and adequate processing time is critical for maximizing amino acid escape from the rumen to the small intestine. It is important to note that some amino acids are inherently more susceptible to ruminal microbial degradation depending on the feed type and processing characteristics, while others demonstrate greater resistance. For instance, lysine, methionine, and phenylalanine exhibited reductions in some feedstuffs and processing methods, whereas other amino acids such as threonine, glutamic acid, and glycine showed variable reductions or increases. Nevertheless, most studies report a significant overall reduction in Total Essential Amino Acids (TEAA) across various processing methods. Among the methods analyzed, electron beam irradiation has proven to be a practical approach for modifying absorption sites and improving the degradability of cottonseed amino acids due to its rapid processing, minimal feed damage, and cost-effectiveness.

In a study by Ghanbari et al. on canola meal, gamma ray and electron beam irradiations at doses of 25, 50, and 75 kGy significantly reduced the ruminal degradability of amino acids, with gamma irradiation showing greater effectiveness—a finding not aligned with our current results. Conversely, Ghanbari et al. found that a 20 kGy electron beam treatment on sunflower seeds reduced ruminal amino acid degradability more than other treatments, supporting our present findings. Additionally, Ghanbari et al. compared 75 kGy gamma ray and electron beam on cottonseed, observing that both methods significantly reduced ruminal amino acid degradability after 16 hours of incubation, with electron beam being more effective, consistent with our findings.

The provision of absorbable amino acids from dietary sources hinges on their escape from ruminal degradation and subsequent intestinal digestibility. Current nutritional systems estimate the amount of amino acids reaching the small intestine based on the Rumen Undegradable Protein (RUP) content and the amino acid profile of the primary feed protein. However, methodological or practical limitations exist, as most estimates are derived after 12 or 16 hours of ruminal incubation, during which samples may be contaminated with microbial amino acids from the rumen, potentially affecting the accuracy of dietary supply estimates.

Ionizing radiation, similar to thermal processes, acts as a physical denaturant of proteins and has been more effective in reducing the ruminal degradability of feed protein and amino acids compared to thermal processes. Success with this method hinges on the controlled application of ionizing radiation energy, such as gamma ray and electron beams, to enhance the nutritional value of feed.

Depending on the intensity and duration of heat, thermal irradiation induces various biochemical reactions between proteins, leading to the cross-linking of polypeptide chains resistant to proteolytic enzymes, thereby reducing their ruminal degradability. This reduction is attributed to the spatial hindrance caused by the cross-linking of polypeptide chains. These reactions primarily occur between lysine and the amide groups of aspartic acid, alanine, and glutamic acid. Lysine, arginine, methionine, and cysteine are more heat-sensitive than other amino acids. Overall, physical processing methods have demonstrated significant potential in altering and reducing the degradability of feed sources.

CONCLUSION

The results of this study demonstrate that all treatments increased the crude protein content of scottonseed while reducing the amounts of Neutral Detergent Insoluble Fiber (NDF) and Acid Detergent Insoluble Fiber (ADF). Furthermore, the dry matter degradability in samples treated with ionizing radiation (gamma ray and electron beams at doses of 20 and 40 kGy) and microwave processing for 3 minutes decreased at passage rates of 2% and 5% per hour. All treatments also reduced the effective dry matter degradability at an 8% per hour passage rate. Additionally, all processing methods in this experiment increased the digestibility of dry matter, organic matter, and organic matter in dry matter. Electron beam, microwave, and roasting treatments reduced ruminal amino acid degradability more than gamma irradiation. The essential amino acids isoleucine, leucine, lysine, and histidine exhibited greater reductions in degradability than other essential amino acids. Among non-essential amino acids, arginine, tyrosine, and asparagine showed the highest reduction in ruminal degradability.

Overall, the findings of this research illustrate that processing methods involving gamma ray and electron beam, as well as thermal methods such as microwave and roasting, not only reduced the ruminal degradability of crude protein and amino acids but also enhanced the *in vitro* digestibility of feed components, thereby improving the nutritional value of cottonseed. Among these methods, electron beam, followed by microwave and roasting, proved most effective in reducing ruminal degradability and enhancing *in vitro* digestibility of cottonseed. Therefore, the processing methods used in this experiment are recommended for improving the quality of cottonseed.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

This study was financed by the Islamic Azad University, Shahr-e-Qods Branch of Agricultural Sciences and Natural Resources, Iran. Authors would like to thank of the National Institute of Animal Science in Karaj, Iran. The Cottonseed was conducted at the Radiation Application Research Institute Atomic Energy Organization of Iran.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

FUNDING

There is no funding for this paper.

DATA AVAILABILITY

Data will be made available on request.

ACKNOWLEDGMENTS

Authors are grateful to the Yazd radiation processing center, Nuclear Science and Technology School, Atomic Energy Organization of Iran for the irradiation operations. This study was financially supported by Shahr-e-Qods Branch of the Islamic Azad University.

REFERENCES

1. Akbarian A, Ghorbani G, Khorvash M, Shawrang P, Dehghan Banadaky M, et al. (2010) Production response of Holstein lactating cows to roasted or electron beam irradiated whole soybean. J Dairy Sci.
2. Alberti A, Bertini S, Gastaldi G, Iannaccone N, Macciantelli D, et al. (2005) Electron beam irradiated textile cellulose fibres: ESR studies and derivatisation with glycidyl methacrylate. Eur Polym J. 41:1787–1797.
3. AOAC (2005) Official methods of analysis. Assoc Off Anal Chem, Washington, DC, USA.
4. ASTM (1984) Method for using the Fricke dosimeter to measure absorbed dose in water. ASTM Standard E1026.
5. Bahraini Z, Salari S, Sari M, Fayazi J, Behgar M (2017) Effect of radiation on chemical composition and protein quality of cottonseed meal. Anim Sci J. 88:1425–1435.
6. Castro SB, Phillip LE, Lapierre H, Jardon PW, Berthiaume R (2007) Ruminal degradability and intestinal digestibility of protein and amino acids in treated soybean meal products. J Dairy Sci. 90:810-822.
7. Ciesla K, Roos Y, Gluszewski W (2000) Denaturation processes in gamma irradiated proteins studied by differential scanning calorimetry. Radiat Phys Chem. 58:233–243.
8. Ebrahimi SR, Nikkhah A, Sadeghi AA (2010) Change in nutritive value and digestion kinetics of canola seed due to microwave irradiation. Asian Australas J Anim Sci. 23:347–354.
9. Ebrahimi SR, Nikkhah A, Sadeghi AA, Raisali G (2009) Chemical composition, secondary compounds, ruminal degradation, and *in vitro* crude protein digestibility of gamma irradiated canola seed. Anim Feed Sci Technol. 151:184–193.