

The effects of radiation and extrusion on patterns of protein subunits and chemical composition of corn and sorghum

Mehdi Moghaddam¹, Parvin Shawrang², Ali Asghar Sadeghi^{1*} and Mohammad Chamani¹

¹Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran, Iran

²Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran

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Abstract

The aim of this study was to determine the effects of gamma radiation and extrusion on chemical composition and electrophoresis of protein subunits patterns of corn and sorghum grains. Samples were extruded and irradiated at doses of 15, 30, and 45 kGy with gamma-rays. The results revealed that ash concentration was significantly high in corn and sorghum irradiated with 45 kGy and 30 kGy respectively. Ether extract and neutral detergent fibre was significantly high in extruded sorghum. Acid detergent lignin was significantly high in sorghum irradiated with 45 kGy. The results of this study showed that the gamma irradiation had the greatest impact in reducing corn and sorghum protein subunits, and the gamma radiation link the subunit of proteins which this characterize can be a good substitute for corn and sorghum in animal nutrition. The results of the present experiment concluded that extruding and gamma irradiation processing be reduced protein subunits MWs and could create positive effects on animal nutrition.

Keywords: Corn, Extruding, Gamma, Sorghum

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Introduction

Gamma radiation and extrusion are physical processing that have positive effects in increasing digestibility of protein in poultry nutrition. The electrophoresis of the radiated proteins showed that the low dose of gamma ray reduced the hybrid force and minor fracture in polypeptide chains and caused the formation of molecules with low molecular weight (MW). Radiation increases the digestibility of through creating cross links, denaturing proteins, and gelatinizing starch (Davies, 1998). Gamma radiation

creates oxidative stress and biomolecular effects by conformational changes, oxidation, rupture of covalent bonds, and formation of free radicals (Cheftel et al., 1985). Hydroxyl (HO[•]) and superoxide anion (O₂^{•-}) are radicals which are produced by gamma ray and are capable of improving the molecular qualities of proteins and lipids, and cause the oxidation and peroxidation of proteins and fats (Halliwell and Gutteridge, 1989). Gamma radiation causes chemical changes in protein. These changes involve its fragmentation, interaction, and oxidation which are created by oxygen radicals produced by water radiolysis (Cho and Song, 2000).

***Corresponding author:** Ali Asghar Sadeghi, Department of Animal Science, Science and Research Branch, Islamic Azad University, Tehran, Iran; E-mail: aasdghe@gmail.com;
Tel.: +98 9195579663; Fax: +98 21 446 4061

Extrusion is a hydrothermal processing which is used as a combination of pressure, moisture, and heat with different times for improving physical building and nutritional value of feed (Fallahi et al., 2013). Extruder is also a technical performance through which the feed under the consistent increase of pressure is processed, extruded, and cooked. Then, it is expanded due to the sudden pressure drop. The impact of thermal processing on degradation of protein and starch of corn has been reported recently Moghaddam et al. (2016).

Valuable information can be obtained about the effect of gamma radiation and extrusion on the quality of feed protein through using SDS-PAGE electrophoresis technique. Thus, the current research aimed at determining the appropriate and precise dose of radiation and investigating the mechanism of improving protein quality in feeds radiated with gamma ray and extruded processing.

Materials and Methods

Chemical analyses

Samples were analyzed for dry matter, ash, ether extract and crude protein were determined by method of AOAC (2000), and also, Crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF) condensation were found according to instruction of Van Soest et al. (1991).

Sample preparation, irradiation and extrusion

Samples were provided by the Market, Tehran, Iran. The whole fat-free samples used in this study were assayed at 893 and 914 g DM/kg for corn and sorghum, respectively. This value was determined by oven drying of 1g sample in duplicate prior to processing. Three paper packages of each sample were irradiated in a gamma cell for average doses of 15, 30 and 45 kGy. One package was placed at a room temperature for use as control. After completing the irradiation, the samples were collected and stored in plastic bags for experiment. Gamma irradiation was carried out in the Radiation Applications Research School, Atomic Energy Organization of Iran by a Gamma-Cell (Co-60), GC-220 (AECL, 1984) research irradiator at room temperature. The dose rate determined by Fricke dosimetry was 2.48 Gy/s (Holm and Berry, 1970). Also, one part of the feed was processed by extrusion at the temperature of 130°C and under pressure and humidity. It was removed from the device within 10-15 seconds.

Monitoring protein subunits pattern

Protein subunits were fractionated by a SDS-PAGE technique according to the Laemmli (1970) method as described by Sadeghi and Shawrang (2008). Electrophoresis device was Vertical Slab Unit, MOD: VSS1100 double gel with cooling chamber, built by

Akhtarian Company, Iran. Electrophoresis of proteins was performed on 12% resolving gel (1.0 mm × 110 mm × 140 mm) with 1.95% acrylamide stacking gel. The gels were kept at a constant current of 30 mA and variable voltage until the Bromo Phenol Blue marker dye reached the bottom of the gel.

Statistical analysis

The collected data were analyzed with a completely randomized design through the general linear model procedure of SAS (for Windows version 9.3). When a significant difference was found, the means were separated by Duncan's multiple range tests.

Results

The effects of processing on chemical composition

The radiation and extrusion had significant impacts on chemical compositions of the experimental treatments ($P<0.05$). The results revealed that ash concentration was significantly high in corn and sorghum irradiated with 45 kGy and 30 kGy respectively. Ether extract and neutral detergent fibre was significantly high in extruded sorghum. Acid detergent lignin was significantly high in sorghum irradiated with 45 kGy.

As can be seen in Table 2, MW of subunit 1 and 2 of globulin were significantly ($P<0.05$) high in corn radiated with dose of 45 kGy while MW of subunit 3 was significantly ($P<0.05$) low. Similarly, MW of subunit 1 of prolamine was significantly ($P<0.05$) high in corn radiated with dose of 45 kGy. The MW of albumin subunit 1 was significantly ($P<0.05$) low in corn radiated with 15 and 20 kGy.

The MW of sub unit 1 of napin was significantly ($P<0.05$) high extruded sorghum and sorghum radiated with a dose of 15 kGy. The MW of subunit 3 of napin was significantly high in sorghum radiated with dose of 45 kGy.

The effects of processing on electrophoretic profiles

The effects of gamma radiation and extrusion processing on the subunits of the proteins of the treatments through SDS-PAGE electrophoresis technique of corn and sorghum are presented in Figure 1. The accumulation of bonded proteins is observable on the upper part of the gel. These bonds were narrower in control and extruded treatments. According to running gel analysis, the accumulated proteins were observable in certain parts of the bonds of the mentioned gels which were narrower and sharper in some samples. In general, these bonds were thicker and clearer in radiated treatments than control and extruded ones. In addition, there were differences in the bonds of the radiated treatments in the observed subunits in the middle of the gel.

Table 1: Chemical composition of corn and sorghum (%DM)

Treatment	DM	OM	Ash	CP	EE	CF	NDF	ADF	ADL
A	89.3	88.4	0.9 ^b	8.887	6.1	1.2 ^a	7.766 ^a	0.69	0
B	90.2	89.3	0.8 ^b	9.594	6.573	0.6 ^b	6.4 ^b	0.67	0
C	89.5	88.7	0.7 ^b	7.969	6.4	0.2 ^c	1.0 ^d	0.67	0
D	89.7	88.8	0.9 ^b	7.721	8.7	0.2 ^c	1.7 ^c	0.67	0
E	89.3	87.9	1.2 ^a	7.933	10.8	0.2 ^c	1.3 ^{cd}	0.67	0
SEM	0.0822	0.0821	0.0019	1.2674	0.5681	0.1264	0.3473	0.0769	0
F	91.4	89.5	2.0 ^b	9.792	7.1 ^b	2.0	12.2 ^b	3.25	0.25 ^b
G	93.7	91.4	2.2 ^b	11.467	9.6 ^a	2.8	20.0 ^a	3.25	0.25 ^b
H	92.2	90.3	1.8 ^b	10.266	4.3 ^d	2.0	9.75 ^c	2.0	0.25 ^b
I	92.3	85.8	3.5 ^a	9.983	5.3 ^c	2.0	8.5 ^d	2.0	0.25 ^b
J	91.3	88.9	2.4 ^b	9.771	6.6 ^b	2.0	8.5 ^d	2.75	0.75 ^a
SEM	0.0676	0.0684	0.0054	1.2339	0.4266	0.8820	0.5554	0.7231	0.0921

DM=dry matter, OM=organic matter, CP=crude protein, EE=ether extract, CF=crude fiber, NDF=neutral detergent fiber, ADF=acid detergent fiber, ADL=acid detergent lignin, SEM=standard error of the mean. Means in the same column do not significantly differ ($P>0.05$); A: Corn, B: Extruded corn, C: Corn irradiated with dose 15 kGy, D: Corn irradiated with dose 30 kGy, E: Corn irradiated with dose 45 kGy, F: Sorghum, G: Extruded sorghum, H: Sorghum irradiated with dose 15 kGy, I: Sorghum irradiated with dose 30 kGy, J: Sorghum irradiated with dose 45 kGy.

Table 2: Estimated MWs of corn protein subunits

Protein	Subunit	A	B	C	D	E	SEM
Globulin	1	65.2 ^a	59.5 ^c	62.7 ^b	65.2 ^a	66.0 ^a	0.456
	2	36.7 ^b	36.9 ^b	37.7 ^b	36.9 ^b	40.2 ^a	0.543
	3	30.9 ^a	30.7 ^a	29.7 ^{ab}	30.0 ^{ab}	29.1 ^b	0.64
Prolamine	1	20.4 ^b	20.7 ^{ab}	20.9 ^{ab}	21.4 ^{ab}	22.0 ^a	0.78
Albumin	1	15.7 ^a	15.7 ^a	14.7 ^b	14.6 ^b	15.5 ^{ab}	0.49

A: Corn, B: Extruded corn, C: Corn irradiated with dose 15 kGy, D: Corn irradiated with dose 30 kGy, E: Corn irradiated with dose 45 kGy.

Table 3: Estimated molecular weight of sorghum protein subunits

Protein	Subunit	F	G	H	I	J	SEM
Napin	1	56.7 ^b	63.0 ^a	63.9 ^a	56.7 ^b	57.4 ^b	0.52
	2	35.9	36.3	36.3	36.1	36.5	0.78
	3	21.5 ^b	21.6 ^b	21.8 ^{ab}	22.2 ^{ab}	22.6 ^a	0.498

F: Sorghum, G: Extruded sorghum, H: Sorghum irradiated with dose 15 kGy, I: Sorghum irradiated with dose 30 kGy, J: Sorghum irradiated with dose 45 kGy.

The molecular weight of the protein subunits of corn are presented in Table 2. Regarding the bonds of Figure 1, it was found that radiation with 15 kGy dose of gamma ray had the best performance in reducing all subunits. The 30 kGy dose of gamma ray had almost the same effects on MWs as extrusion and their MWs are approximately equal to that of control treatment. However, the 45 kGy dose of irradiation increased the MWs.

The MWs of protein subunits of sorghum are shown in Table 3. As it shown in Figure 1, radiation with 30 kGy dose of gamma ray had the best performance among the processing in decreasing all subunits. The 15 kGy dose of gamma ray had almost the same effects on MWs as extrusion. However, the 45 kGy dose of radiation increased the MWs of subunits 2 and 3 of napin protein. Radiation with 15 kGy dose of gamma ray increased the MWs of subunit 1 of protein in sorghum.

The effects of processing on electrophoretic profiles

The effects of gamma radiation and extrusion processing on the subunits of the proteins of the

treatments through SDS-PAGE electrophoresis technique of corn and sorghum are presented in Figure 1. The accumulation of bonded proteins is observable on the upper part of the gel. These bonds were narrower in control and extruded treatments. According to running gel analysis, the accumulated proteins were observable in certain parts of the bonds of the mentioned gels which were narrower and sharper in some samples. In general, these bonds were thicker and clearer in radiated treatments than control and extruded ones. In addition, there were differences in the bonds of the radiated treatments in the observed subunits in the middle of the gel.

The molecular weight of the protein subunits of corn are presented in Table 2. Regarding the bonds of Figure 1, it was found that radiation with 15 kGy dose of gamma ray had the best performance in reducing all subunits. The 30 kGy dose of gamma ray had almost the same effects on MWs as extrusion and their MWs are approximately equal to that of control treatment. However, the 45 kGy dose of irradiation increased the MWs.

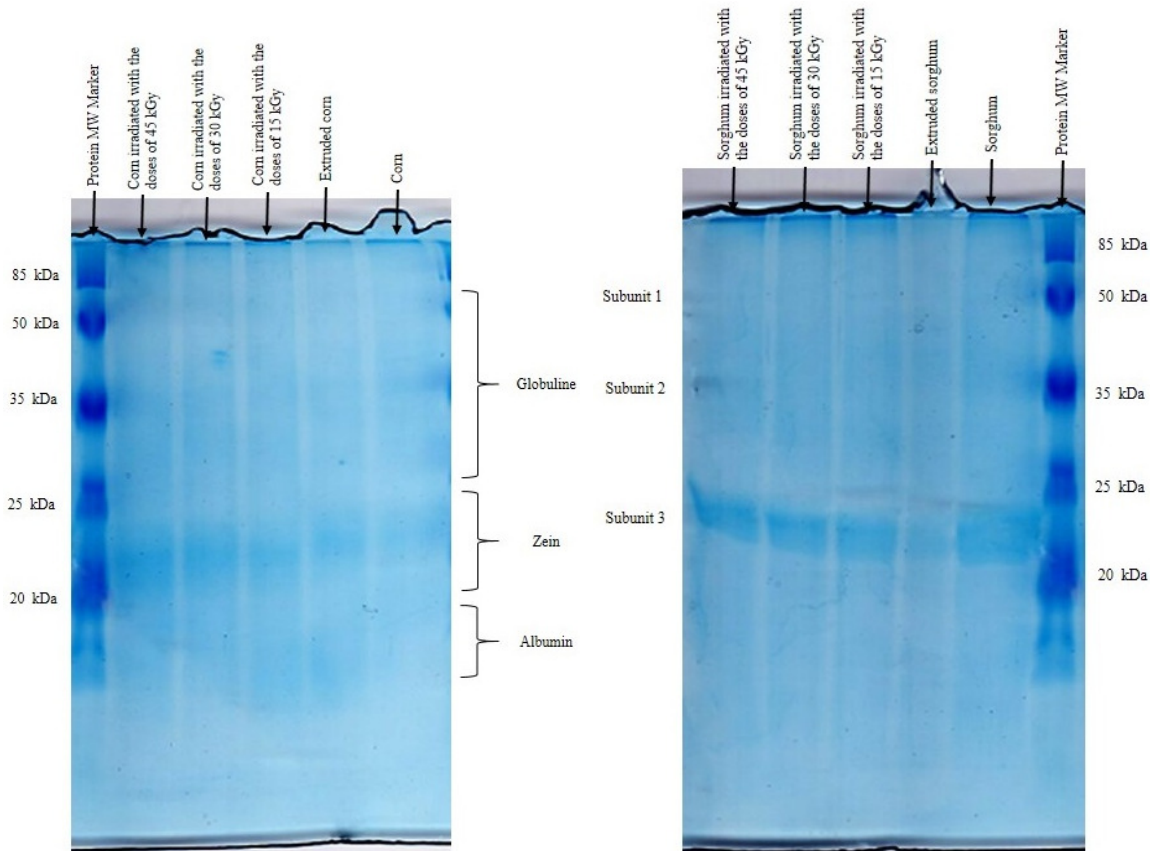


Fig. 1: SDS-PAGE electrophoretic patterns of corn (left) and sorghum (right) seeds

The MWs of protein subunits of sorghum are shown in Table 3. As shown in Figure 1, radiation with 30 kGy dose of gamma ray had the best performance among the processing in decreasing all subunits. The 15 kGy dose of gamma ray had almost the same effects on MWs as extrusion. However, the 45 kGy dose of radiation increased the MWs of subunits 2 and 3 of napin protein. Radiation with 15 kGy dose of gamma ray increased the MWs of subunit 1 of protein in sorghum.

Discussion

The data of the present study were in line with previous findings on chemical compositions of corn and sorghum (Rousta, et al. 2014; Shawrang et al., 2011, 2013; Moghaddam et al., 2016).

Thermal processing can denature proteins and probably change proteins into a resistant structure, mostly enzymes (Sadeghi and Shawrang, 2006). The formation of gel or regular protein complexes reduce the accessibility of chemical groups for the action of enzymes of proteolytic microbes and finally reduce the degradability speed and extent of proteins, especially glycinin (Moghaddam et al., 2016).

Flaviyo and Apenten (1997) reported that thermal processing causes structural changes in proteins and increases hydrophobicity on the surface of proteins. The increase in hydrophobicity of protein is caused due to the separation hydrogen bonds and other weak non-covalent bonds and, consequently, denaturing of proteins and changing the position of amino acids.

The transmission of hydrophobic amino acids from inside to the surface of molecules causes the hydrophobicity of molecule surface and decreases its accessibility for proteolytic enzymes (Folawiyo and Apenten, 1997). Due to the increase in surface for the action of enzymes in protein structure, extruding through denaturing of proteins increases their digestibility. Most of the anti-enzymatic factors lose their activity because of denaturing through extrusion.

Like heat, ionizing radiation is one the physical factors of denaturing the protein. These kinds of radiations in juicy feed ionize water molecules to activated ions and make physicochemical changes in proteins and denature them by producing peroxide (Cieřla et al., 2000). Denaturing increases the hydrophobicity of proteins (Shawrang et al., 2008) and therefore the solubility of proteins get reduced. Denaturing of proteins causes the exposure of

hydrophobic amino acids. Considering that the side groups of hydrophobic amino acids are the chemical actives for pepsin, trypsin and chymotrypsin enzymes, radiation provides suitable conditions for more activity of these enzymes in intestine (Abu et al., 2006). Researchers have stated that the amount of free radicals produced in a matter under radiation is directly proportional to the radiation doses. Increasing the radiation dose will increase its effects on denaturing process (Siddhuraju et al., 2002).

The electrophoresis studies on polyacrylamide gel indicated that radiation opens the polypeptide chains and then sticks them together (Landry and Moureaux, 1981; Lee et al., 2001). Radiation creates non-covalent bonds between free amino acids as well as peptides and proteins in the solution (Garrison, 1987).

The radiated feed contained more crude protein and less MWs than the control treatment since gamma radiation creates oxidative stress and bimolecular effects through conformational changes, oxidation, rupture of covalent bonds, and formation of free radicals (Cheftel et al., 1985).

Radiation breaks hydrogen bonds and denatures protein (Lacroix et al., 2002), changes the conformation of protein, and increases the hydrophobic sites on the surface of protein. There are lots of hydrophobic amino acids inside the protein molecule in inseparable structure of globular proteins. Ion radiation can increase flexibility and denaturing of proteins, reveals the already blocked non-polar groups, and increases the hydrophobicity of the surface of the protein (Garrison, 1987). This is an acceptable condition for protein so that they can approach each other and form a complex (Lacroix et al., 2002). There are evidences for the existence of these reactions by electrophoresis technique (Shawrang et al., 2013). The impacts of gamma radiation on subunits of protein through electrophoresis technique showed that radiation stuck the protein and created some stains in the beginning of the gel (Shawrang et al., 2013), while, these samples did not exist in unirradiated samples.

Ionizing radiation decreases the accessible solubility of proteins and causes the rupture of the regular structure of molecules of the protein, decomposition, cross-linking, and sticking of the polypeptide chains together through reducing the enthalpy of protein denaturing and making physicochemical changes (De Boland et al., 1975). In this way, they make changes in the MWs of the subunits of protein. These explanations may also be responsible for the results of the present study.

Conclusion

The results of the present study showed that radiation and extrusion had significant in chemical compositions of the treatments. Gamma radiation and

extrusion increased the nutritional value and digestibility of feed by making changes in structure of protein and starch. The comparison of the treatments revealed that radiation with 45 kGy dose of gamma radiation provided more acceptable results. The analysis of 12% gel of corn and sorghum seeds revealed that radiation with 15 and 30 kGy doses of gamma ray had the best performance in reducing all subunits.

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