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Effect of Radiation Dose, Dose Rate and Annealing Temperature on Edible Potato Starch by Spectroscopic Methods

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Abstract

Potato starch (PS) is a favorite food choice in different forms. During its storage and applications, the PS is exposed to different radiation. In the present study the authors attempt to compare radiation effects (gamma and Electron beam of same radiation dose) in PSby Electron spin resonance (ESR) and Fourier transform infrared (FTIR) methods. ESR spectra of gamma irradiated PS appeared to have resolved shapes than the electron beam (EB) irradiated counter parts. The spectral changes are thought to arise due to changes in crystallinity, crystalline structure and amylose content of starch molecule due to irradiation. Free radicals produced in PS (gamma and EB) are identified by recording ESR spectra. Computersimulations are used to analyze ESR spectra and the data suggest presence of at least three types of free radicals (radical I radical II and radical III), formed the by cleavage of 1 - 4 and 1 - 6 glucosidic bonds of starch molecule. Formation of chain cleavages and crystalline changes are confirmed by the FTIR data. Free radical concentration increased linearly (gamma and EB) with radiation dose suggesting the possible use of PS for dosimetric applications. The ESR line shape and spectral features almost remain same up to elevated temperature suggesting the ideal qualities of PS dosimeter. The data further suggest that free radicals produced in PS have high thermal stability and have an activation energy of (85 K J /mole) as evaluated from Bloch analysis.

Keywords: Gamma radiation, Electron beam (EB), ESR, FTIR, radiation dosimeter, Bloch analysis, potato starch (PS).

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INTRODUCTION

Potato Starch (PS) is one of the important food material consumed all over. The increase in health and safety concerns regards chemical modification of PS has caused concern and interest among researchers in its modification by physical techniques [1-3]. Gamma radiation is a tool to alter chemical structure of different types of starches inducing changes in chemical as well as physical properties [4]. Corn starch is irradiated with gamma radiation to a dose of 1,5,10, 15, 50 K Gy and resultant effects are investigated [5]. The studies indicate increase of amylose content at 10 K Gy radiation dose. But for remaining radiation doses amylose content, gelation properties are low and reduction in pasting properties is observed, as a result of cross linking. On irradiation crystalline properties of starch are effected; however its thermal properties are less influenced [6]. Gamma radiation exposure of starch caused fragmentation of molecules through the cleavage of glucosidic bonds producing free radicals resulted in reduction of amylose content and degree of crystallinity [7]. Radiation exposure induce Visible and significant changes in granular structure of starch producing cracks. Effect of gamma Irradiation in potato starch in the radiation dose range of 0 - 15 K Gy is reported [8]. The studies suggest increase of Yellow color parameter and decrease of viscosity with radiation dose, indicating degradation of starch. Hager et al [9] have compared gamma irradiation effects (radiation dose of 3,5,10,25 K Gy) in wheat strach (WS) and potato starches(PS) and observed the decrease of peak gelatinization temperature, swelling ability, relative viscosity and textural properties with radiation dose. In contrast, apparent amylose content, swelling power have increased. Shape and size of PS granules is more sensitive to sheer at high radiation doses. When compared to the WS, the granular stress was more in irradiated PS.

Cassava starch is irradiated to a dose range of 1 – 10 K Gy and resultant effects are investigated [10]. The results indicate that micro structure of starch granules is not much influenced by irradiation. But deformation and fragmentation of granules is observed, which resulted in increase of water solubility. Cleavage of Glucosidic bonds and formation of smaller molecules is reported. Effect of gamma irradiation combined pyrrolysis on digestible fraction, physico – chemical and molecular structure of starch has been studied by Nguyen [11]. Pyrrolysis led to decrease of indigestible fraction (IDF), while radiation treatment led to increase of IDF. The combined action of pyrolysis and irradiation resulted in the interaction of functional groups as well as structural groups. Gamma irradiation effects in starch have been reviewed by Xiangli [12], who proposed mechanism for radiative degradation of starch. As per this mechanism, the water molecule present in starch dissociate into H and OH radicals. The OH radicals interact with starch molecules producing free radicals and water. The free radicals cross link to form networks structures resulting in enhancement of viscosity. The other path of reaction suggest interaction of free electrons with starch producing free radicals, which also cross link to form polymer networks. Gamma irradiation and annealing of sago starch (SS) was reported by Lee [13] in the radiation dose range of 5, 10,15, 25, 50 K Gy. Two SS samples namely the sample annealed at a temperature below 5°C of gelatinization temperature (T-5) and the other one below 10°C to the gelatinization temperature (T -10). For both the samples variations in the amylose content, Phenyl carbonyl content, ratio of FTIR absorption bands (R 1047 /R1022), swelling power, solubility, thermalbehaviour pasting property, morphology are investigated. Annealing starch at T-5 produced more crystalline regions compared to T-10 at 25, 50 K Gy radiation dose.

Bruna et al have [14] have compared the gamma irradiation effects in corn (CS) and potato starch (PS). The samples are irradiated to 1-15 K.Gy dose at a dose rate of 1 K.Gy /hr. The studies suggest that granular size varies but morphology not effected. Change in intensity of FTIR absorption bands 2000 – 1500 cm^{-1} are observed for PS but not for CS. Irradiation effected colour parameter but light permeation property not changed markedly. The XRD patterns are much influenced by irradiation. Effect of gamma irradiation on starch granular structure and physico - chemical properties of brown rice starch has been reported [15]. SEM micro graphs indicate granular surface was not influenced with retention of poly hedral shape. The XRD pattern suggest A type crystalline pattern with decrease of Degree ofcrystallinity DOC from 22.53 to 20.4%, upon irradiation. With regard to pasting properties pasting temperature decrease with increase of radiation dose. The transition temperature and enthalpy, apparent amylose content decreased with decrease of radiation dose.

Dual modification approach of tropioca starch using gamma radiation and carboxy methylation was reported Imtiaz et al [16]. The CS is simultaneously exposed to gamma radiation dose of 25, 35, 45, 60 K Gy as well as different degrees of carboxy methylation. Resultant changes are monitored by FTIR, SEM, TGA, XRD techniques. Dual modification caused enhancement in thermal stability change of morphology and decrease of DOC (Xc). The modified starch is used for several applications in oil, gas and textile applications. Effect of gamma irradiation on the technological properties of starch mixed with turmeric derived from primary and secondary ohizomes is reported [17]. The compounds are exposed to 2, 3, 5, 10 K Gy radiation dose and changes are analyzed by SEM & XRD, thermal methods. Swelling power and solubility in water were also investigated.

Morphology of the granule was not influenced but molecular structure was not changed. The crystalline pattern was observed to be B-type. The DOC was decreased with radiation dose except at 5 K Gy radiation dose. The gel temperature was not effected but gelatinization enthalpy (Delta H) was decreased. The water solubility increased; while swelling power reduced. The modified samples could be used in food applications.

Gamma and electron beam radiation of starch is a popular tool to alter its chemical structure making it to suit for different applications [18-24]. In spite of the studies, focus is laid on the following aspects in the present studies.

1. Though formation of free radicals is reported, their identification, thermal stability and radiation dose dependence, dose rate effects are not comprehensive.
2. Identification of free radicals in irradiated PS is considered to be an important task due to their spurious effects on human health.
3. The electron spin resonance (ESR) technique is used to identify the free radicals under different conditions and computer simulations are used to analyze the spectra.
4. Dosimetric applications of PS is attempted.
5. The ESR results are correlated with the FTIR data.

EXPERIMENTAL

Commercially procured (Sigma – Aldrich) PS samples in white powder form are used in the present studies. Gamma and EB irradiation of PS are carried out using Cobalt -60 (dose rate of 15 K Gy/hr) and Electronbeam accelerators (15 K Gy/ minute) radiation sources at radiation research center BARC Mumbai. The PS samples are characterized by electron spin resonance (to identify free radicals and their behavior under different conditions) and Fourier transform infrared techniques (to elucidate chemical changes occurred due to irradiation). ESR spectra are recorded on Bruker – model spectrometer operating at X – band frequencies and 100 KHz modulation. PS samples with same weight are exposed to gamma and EB radiations to different times to the desired radiation dose. After irradiation the samples are transferred to quartz tubes without any delay under non-oxidized conditions. FTIR spectra of PS are recorded on Perkin – Elmer spectrometer by making the pellets of PS (about 10 – 20 mg) along with potassium bromide. For all the FTIR measurement equal amounts of PS are taken.

RESULTS AND DISCUSSIONS:

Temperature Dependence of Free Radicals

Effect of annealing on free radicals produced in irradiated PS is investigated by recording ESR spectra as shown in Figure 1. With the increase of temperature to 60°C (333 K), the spectral features almost remained the same. On further heating to 120°C (393 K), the intensity of hyper fine pattern decreased and singlet like structure remained. This result is analogous to the earlier observations [25-28]. The overall decrease in intensity of the spectrum is about 30% with the increase of temperature from RT (293 K) to 120°C (400 K). The results suggest that free radicals produced in PS have high thermal stability unlike many synthetic and biopolymers (Poly Carbonate- 100°C [29] Propylene Ethylene Copolymer 105°C [30] – Poly ethylene terepthalate 120°C [31], Polyglycolic acid 100°C [32]. The result further suggest that free radicals produced in semi-crystalline polymers are trapped in both amorphous regions as well as crystalline regions and become reactive up on annealing. The free radicals trapped in amorphous regions decay at low temperatures [29,30]; while those trapped in crystalline regions decay slowly even after heating elevated temperatures as observed in the present studies. Further, the starch molecule consists of several inter and intra molecular hydrogen bonds, which form ladder / network structures, which obstruct free radical reactions making them to be thermal stable.

Assignment of Spectrum

ESR Spectrum of irradiated starch is reported to exhibit two well-known line shapes designated as AA′ and BB′ [25, 26]. The difference between the two line shapes is, AA′ possess some hyperfine structure; while BB′ is a broad singlet. Another difference is AA′ line shape decay at low temperatures than the BB′; their post irradiation stabilities are also different (AA′ decay at low post irradiation time than BB′). The spectrum observed in the present studies is appeared to be a superposition both AA′ and BB′.

Efforts have been made by various authors to assign free radicals (designated as radical I, II, III and IV) responsible for the ESR spectra of different types of irradiated starches [31]. The studies indicate the cleavage of 1→4 and or 1 -6 glucosidic bond glucosidic bond is the main event of irradiation (either EB, gamma or UV) producing radicals I, II, III & IV. Radical (I) abstracts oxygen and convert to corresponding peroxy radical designated as radical (II). Though existence of third type of radical is proposed its structure was not assigned [25-28].

Starch consists of two important molecules namely amylose and amylopectin. Out of the two, the amylose is a linear chain of glucose units connected by 1 – 4 glucosidic bonds. . While amylopectin is branched and built up by glucose units and branched to the main chain through 1 – 6 glucosidic bonds. When starch is exposed to radiation, the main event of radiation attack is cleavage of 1 – 4 glucosidic bond forming radical II and gives component doublet. Radical II undergo internal conversion and or abstraction of proton / hydroxyl proton producing radical III and gives component multiplet. Radical I is generated by the cleavage of 1 – 6 glucosidic bond and gives component singlet / doublet. The designation, chemical structure and g – values of the free radicals are as given Table 1.

Table 1. The chemical structure and g – values of PS.

Designation of free radical	**Chemical structure**	**g-value**	**Type of Hyperfinestructure**
R1	CH(OH) – C (O) – O – C (H) -	2.0044	component quintet /triplet / singlet
R2	- C – C (H) – O – O.	2.0037	component doublet
R3	H C (OH) – C (H) – C H (OH) – O	1.9945	component multiplet

Though the formation of these radicals is predicted, the experimental identification (by ESR) was only emperical. In this context the authors have used computer simulations methods to assgn ESR spectra.

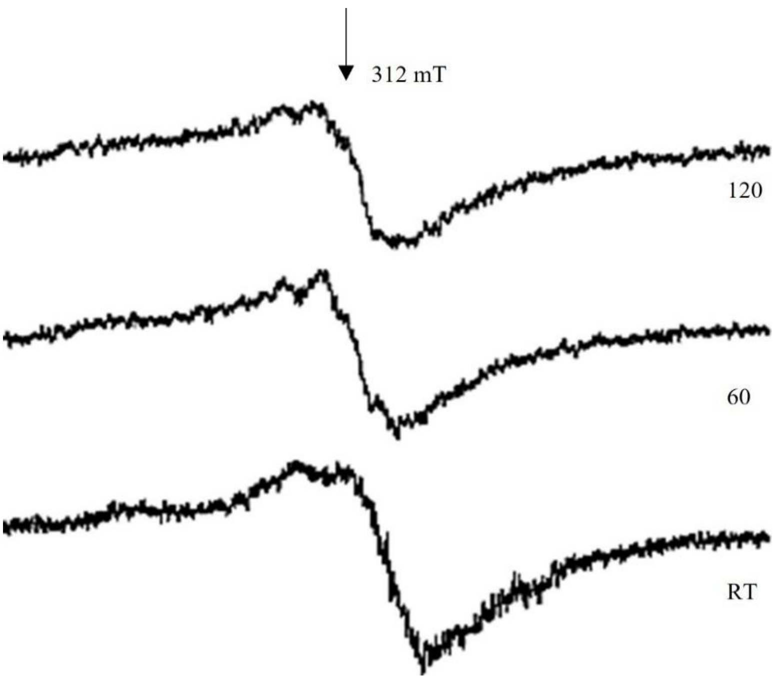

Figure 1. Temperature dependent ESR spectra of PS.

Computer Simulations by Total Curve Fitting Method

ESR spectra of irradiated starch are also analyzed by total curve fitting method (33A). The method involves generation of various component spectra arising due to different free radicals expected to be produced on different chain cleavages. The generated component spectra are superposed in certain proportions to obtain superposed spectra which are to be compared with the experimental spectrum to match the values of intensity against magnetic field. Various magnetic parameters (ni and mi; number of hyperfine lines due to alpha and beta protons, line width (ai), hyperfine splitting constants (Ai and Bi), center of spectrum Xoi) (33B). Component spectra are generated for the free radicals listed in Table 1 as shown in Figure 2. The superposition of component spectra results in the experimental spectrum at RT. Magnetic parameters of the component spectra are listed in Table 2. The simulation data suggest presence of free radicals I, II and III and cleavage of 1 – 4 and 1 – 6 glucosidic bonds. The results are analogous to the earlier reports [25 – 31] and computer simulations theoretically confirm their presence / existence. Due to the cleavages the decrease of amylose content is expected resulting in decrement of crystalline property.

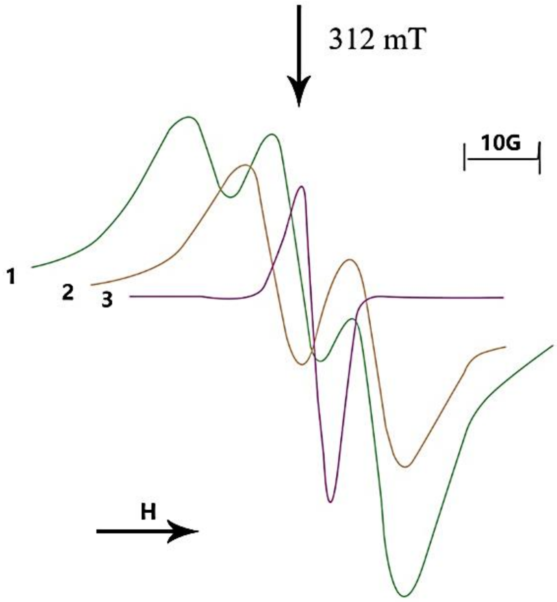

Figure 2. Component spectra of irradiated PS Curve 1 Component multiplet Curve 2 component doublet and Curve 3 Component singlet

Table 2. Magnetic parameters of irradiated PS.

Component	Relative Intensity Y maxi	Line width a I G	Centre of Spectrum X oi	Hyperfine Splitting A_i	B_i	n_i	m_i	radical assignment
1 Multiplet	4	10	3120	13	25	2	5	R3
2 Doublet	9.5	6	3119	10	0	2	1	R2
3 Singlet	10.5	5.5	3121	0	0	1	1	R1

BLOCH ANALYSIS TO EVALUTE ACTIVATION ENERGY

Bloch analysis is used to evaluate activation energy associated with free radical decay of irradiated starch [34]. The method involves calculation of line width and tow (r) are at each temperature (1 / T) (as listed in Table 3) and plot is drawn between them as shown in Figure 3. The values of line widths employed in computer simulations are used for this purpose. From slope of observed straight line the value of activation energy is estimated to be 85 K J / mole.

Table 3. Parameters used for Bloch Analysis.

S.N.	Temperature K	1/T x10^{-3}	τ	1/τ	log (1/τ).
1	300	3.33			
2	330	3.75	0.00758	131.82	2.55
3	420	2.30	0.00690	135.35	2.72

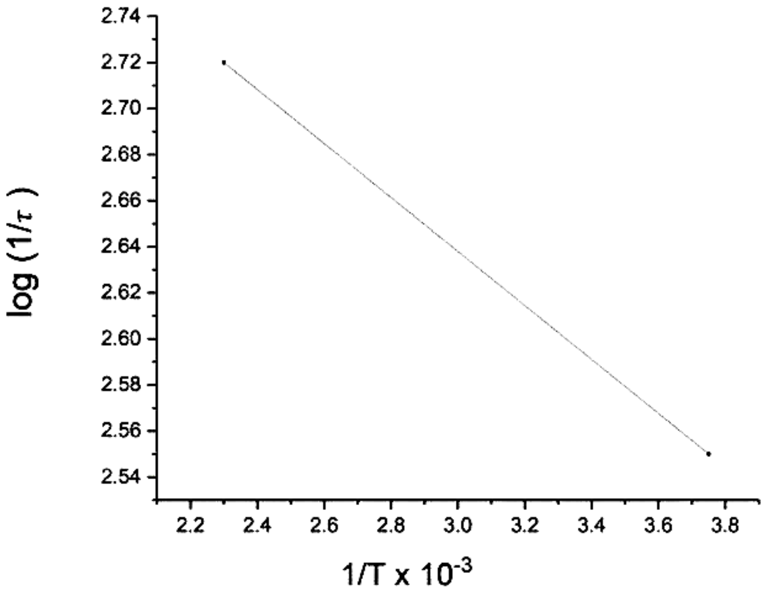

Figure 3. Bloch analysis of PS.

Radiation Dose Dependence of Free Radicals

ESR spectra of PS irradiated with EB to different radiation doses (30. 60, 90 K Gy) are as shown in Figure 4. The spectra is assumed to be superposition of component AA′ and component BB′.

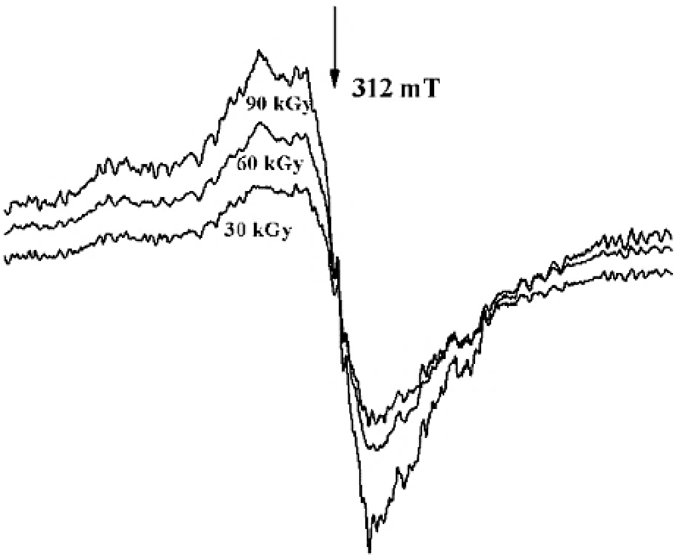

Figure 4. ESR spectra of PS irradiated to different radiation doses.

Free radical concentration is calculated (double integration methods) from the ESR spectra at different radiation doses (30, 60, 90 K Gy) and plot of ESR intensity against radiation dose is drawn as shown in Figure 5 indicating its gradual increase with radiation dose. Therefore free radical formation linearly increase with radiation dose.

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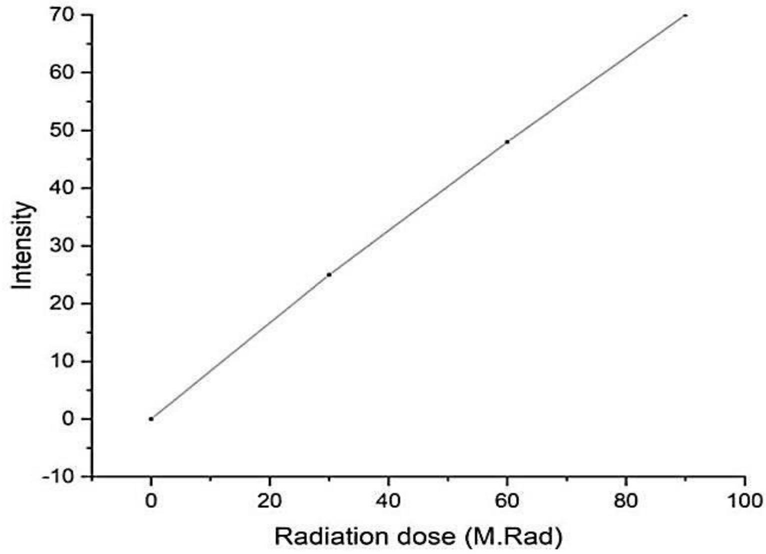
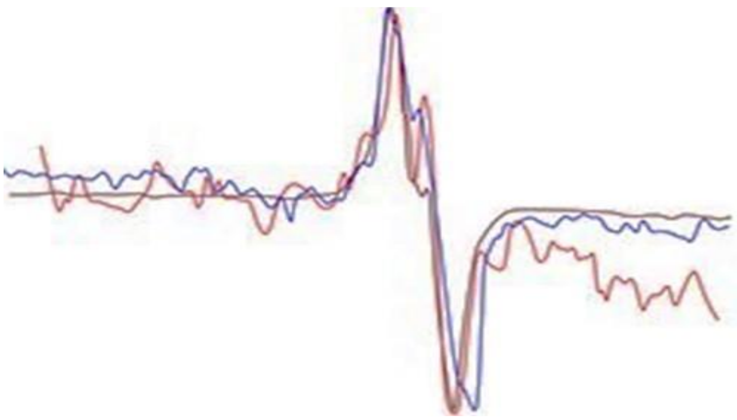


Figure 5. Variation of ESR intensity against radiation dose (EB)].

Effect of Gamma Irradiation: ESR Studies

ESR spectrum of gamma irradiated starch is as shown in Figure 6. The spectrum appears to have weak hyperfine line resembling AA' component. When compared to the EB irradiated PS, the gamma irradiated PS has exhibited resolved hyperfine structure. The spectral differences are in EB and gamma irradiated starch are attributed to the dose rate effects. Starch is considered to a bio-polymer consisting large number of terminal hydroxyl groups, which have a tendency to form hydrogen bonds between the adjacent macromolecular chains and constitute hydrogen bridge/ ladder. The bridge / ladder provide some sort of crystalline structure and act as protective mantle to prevent degradation of starch [35]. Since PS is irradiated to same radiation dose. the gamma irradiation with low dose rate has more exposure time than the high dose rate. Therefore gamma radiation with more exposure time cleave more hydrogen bridges damaging the crystalline structure; whereas the EB with high dose rate and less exposure time is capable of breaking less number of hydrogen bridges. Due to the variations in radiation dose rates and exposure time damage in PS is more making to assume amorphous nature. The PS with more amorphous nature will exhibit resolved hyper fine shapes as observed in present studies. EB irradiated spectra with less damaged crystalline structure will have latent hyperfine shape. However in both cases cleavage of glucosidic bonds production free radicals listed in Table 1 take place. The result is in consequent to the earlier reports of cleavage of 1–4 or 1–6 glucosidic cleavages [36] on irradiation of starch. Effect of high energy radiation exposure of starch results in de - hydroxylation as reported by Falade and Kolawale [37].



1
2
3

Figure 6. ESR spectra of PS irradiated to different radiation doses Curve1 30 K Gy, Curve2 60 K Gy, Curve3 90 K Gy

DOSIMETRIC APPLICATIONS OF PS

Radiation dosimeters are used to measured radiation dose absorbed by materials in different environments. Polymer dosimeters an important class among them, Among polymers bio-polymers like gelatine, starch have advantage for this purpose. In principle any material property linearly varies with absorbed radiation dose, that material can be treated as suitable for dosimetric applications. In the present studies, the ESR intensity linearly varied with radiation dose as shown in Figure 5 and Figure 7 [38]. Therefore PS could be considered for dosimetric applications with ESR as tool.

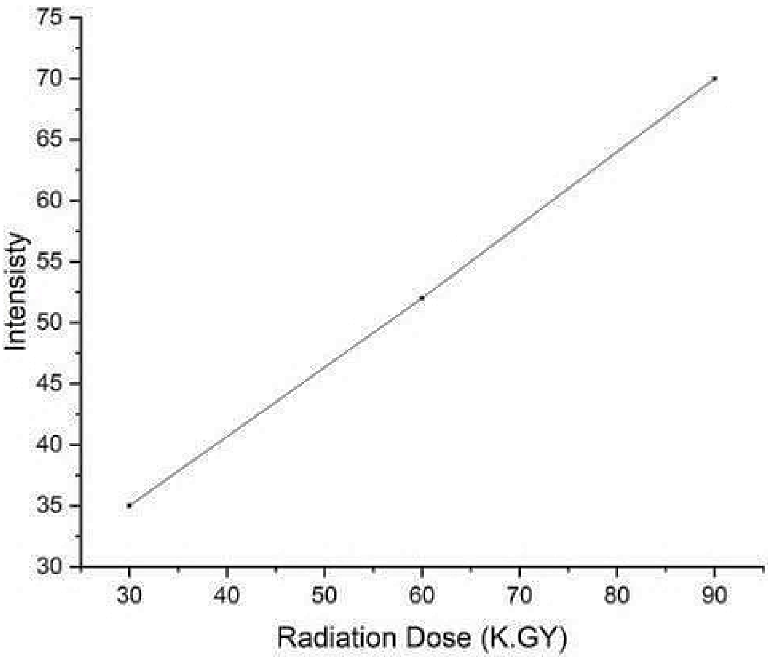


Figure 7. Variation of ESR intensity against Radiation dose [gamma].

FTIR Studies

Green curve 1- 30 K Gy; Indigo curve 2 – 60 K Gy; Violet curve 3 – 90 K Gy;

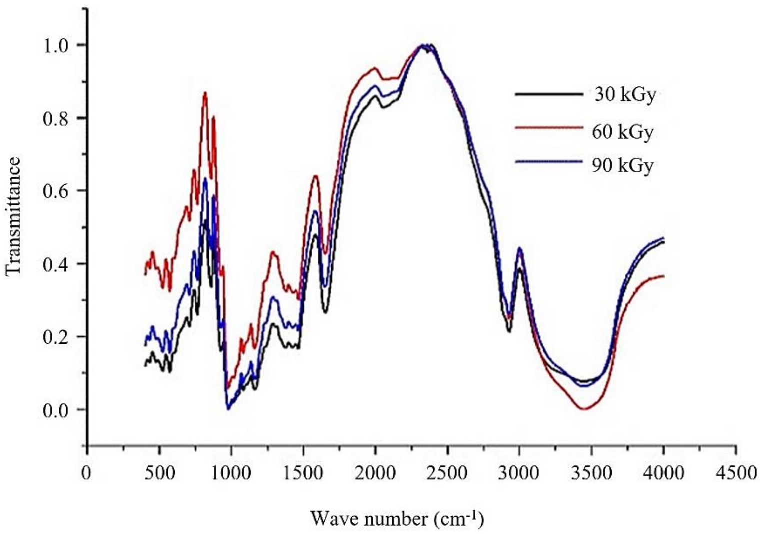


Figure 8. FTIR spectra of PS irradiated to different radiation doses curve1 30 K Gy curve2 60 K Gy curve3 90 K Gy

FTIR spectra are recorded for un-irradiated and irradiated PS to characterize the changes in chemical structure in PS due to gamma and electron beam irradiations. Figure 8 represent radiation dose dependent FTIR spectra (curve 1 – 30 K Gy, curve 2 – 60 K Gy & curve 3 – 90 k Gy). The absorption bands together with their interpretations [36, 37, 39] are listed in Table 4. After irradiations new functional groups were not found and the spectral pattern was similar, except for the intensity of few bands. On irradiation, the following changes are noted,

1. Decrease in intensity of 3700 – 3000 / 3420 cm^{-1} band representing the cleavage of O – H or H - H bands.
2. The band at 1002 cm^{-1} was assigned to the vibration of C-O-H deformation and is also used to express the amorphous nature in starch [36, 37, 39]. On irradiation, it was noticed that the intensity of the band 1002 cm^{-1} increased gradually indicating a decrease in ordered structure and amorphousity of PS. The result is in agreement with the ESR study, where spectral resolution is more due to increase in amorphous content of PS on irradiation. The data further tallied with XRD measuments of PS where decrease in crystalline content decrease on irradiation [15,16].

Table 4. FTIR absorption bands of native and irradiated PS.

S.N.	Band position	Intensity	Assignment	References
1	3700 – 3000 / 3442	Broad & high	O – H & H – H	32,33
2	2920, 2850	Medium	C - H vibrationof CH2 & CH3	32,33
3	1650	Medium	C – H vibrations	32,33
4	1380	Medium	C – H vibrations	32,33
5	1170	Medium	C – O, C – C, C – H Vibrations	32,33
6	1080	Medium	C – O, C – C, C – H Vibrations	32,33
7	1002	Medium	- C – O – H – Deformation	34

FTIR of gamma irradiated PS

FTIR spectra of PS irradiated to 30 K Gy (green line), 60 K Gy (blue line) and 90 K Gy (indigo line) are shown in Figure 9. The spectra are similar to the EB irradiated counter parts band positions are almost same but changes in band intensities have small variations. Particularly the intensity variation of 3400 – 3000 cm-1 band different than the EB irradiated spectra. The variations in band positions is already explained in case of EB irradiated PS [39–41].

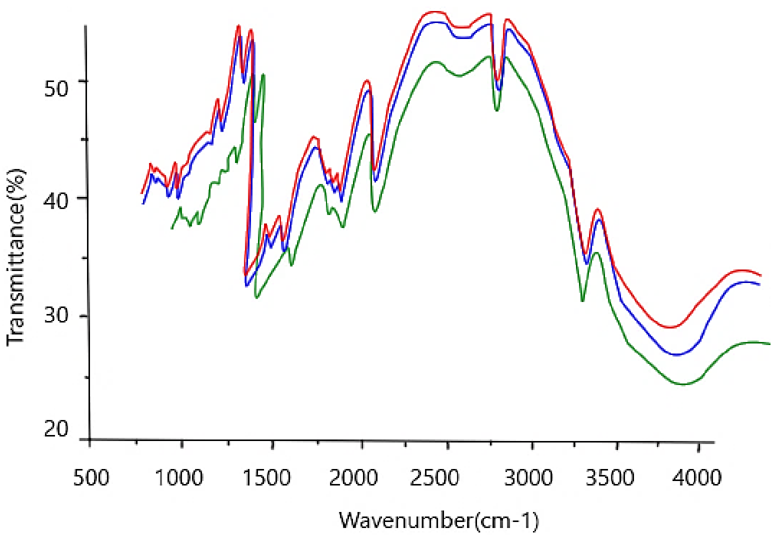


Figure 9. FTIR spectra of gamma irradiated PS at different radiation dosesGreen -- 30 K Gy; Blue -- 60 K Gy; Indigo -- 90 K Gy.

CONCLUSION

In conclusion Gamma irradiation and EB irradiation of PS cause cleavage of 1 – 4 and 1 – 6 glucosidic bonds producing radicals I, II and III. Radical I gives component singlet spectrum; while radicals II and III give component doublet and multiplet spectra. Due to variation in radiation dose rate Gamma irradiated ESR sprctra have resolved shaped when compared to the EB irradiated spectra. Due to low rate and high exposure time Gamma radiation considerably damage crystalline structure increasing amorphous content, increasing spectral resolution. Whereas in case of EB irradiation damage of crystalline content is less and responsible for latent hyperfine structure. Dose dependent ESR spectra indicate the growth of free radicals on irradiation and starch is suitable for dosimetric applications. Temperature dependent ESR studies suggest that free radicals in PS have high thermal stability. Activation energy for decay of free radicals is evaluated from Bloch analysis and it is considerably higher than several other conventional polymers.FTIR studies of irradiation PS confirm damage of hydroxyl bands and crystalline content or increase in amorphous content.

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