Food Bioprocess Technol
DOI 10.1007/s11947-016-1823-x

ORIGINAL PAPER

Impact of Electron Beam on Storage Protein Subunits, In Vitro Protein Digestibility and Trypsin Inhibitor Content in Soybean Seeds

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Received: 9 September 2016 / Accepted: 30 October 2016

Abstract The present investigation was carried out to study the effect of 4.8, 9.2, 15.3 and 21.2 kGy of electron beam (EB)-irradiation on major storage proteins viz. glycinin (11S) and β-conglycinin (7S), in vitro protein digestibility (IVPD), and trypsin inhibitor (TI) content in seeds of three soybean genotypes. Densitometry of SDS-PAGE protein profile revealed significant ($P < 0.05$) reduction in α', α and β subunits of 7S fraction at all doses. This reduction was higher ($P < 0.05$) than the decline observed in acidic and basic subunit of 11S fraction. Basic subunit registered significant ($P < 0.05$) increase at specific doses in two genotypes. All the doses induced significant ($P < 0.05$) increase in IVPD, and the increase due to 9.2, 15.3 and 21.2 kGy was higher ($P < 0.05$) compared to 4.8 kGy dose. The impact on TI content was genotype-dependent. The study showed a higher ($P < 0.05$) decline in the concentration of 7S fraction compared to 11S fraction and improvement in IVPD of soybean seeds due to exposure to EB-irradiation, which may influence the functional and nutritional value of soy products processed from them.

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Keywords Soybean seeds · Electron beam irradiation · Trypsin inhibitor · Storage proteins · IVPD

Introduction

Globally, soybean is the major oilseed crop, with the USA, Brazil, Argentina, China and India as the leading producers. Soymeal obtained after extraction of oil is used as feed for poultry and swine. The food products processed from the whole seeds are part of the traditional diet in countries like Japan, China, Taiwan, Korea, and Malaysia. It is one of the major economical sources of protein (40%) packed with essential amino acids, and rich in biomolecules which can reduce the risk of onset of breast cancer (Messina et al. 1994), diabetes (Nordentoft et al. 2008), cardiovascular diseases (Lichtenstein 1998), and osteoporosis (Potter et al. 1998). Therefore, soy-based products can combat malnutrition in developing countries, and as a 'healthy food' can keep the chronic diseases at bay. The functional and nutritional value of soy products depends upon two major storage proteins, namely, β-conglycinin (7S) and glycinin (11S), which account for more than 70% of the total proteins in soybean seeds. β-Conglycinin (7S) with molecular weight 180,000 Da is composed of three subunits, namely, α', α and β. Glycinin (11S) is a hexameric protein with molecular weight of 360,000 Da, each subunit consisting of acidic and basic polypeptide linked by disulphide linkages. Of the total storage protein, the proportion of glycinin is higher (60%) than β-conglycinin (40%). Glycinin (11S) contains three to four times more sulphur-containing amino acids, particularly methionine, compared to β-conglycinin (7S) fraction. More importantly, 11S fraction has higher water holding capacity and yields larger aggregates suitable for *tofu* making. The ratio of 11S to 7S fraction in soybean seeds affect the functional properties of food products processed from them

Published online: 17 November 2016

(Kim and Wicker 2005; Poysa et al. 2006). Further, soybean seeds suffer from poor protein digestibility, which is attributed to the presence of antinutritional factors like trypsin inhibitor and conformation of native proteins.

Gamma rays employed to disinfest grains, food and feed material including soybean at low doses have been reported to reduce undesirable components in soybean seeds (Diehl 2002). EB-irradiation, which consists of high-energy electrons and does not involve the use of radioactive material employed in gamma rays irradiation, has also been reported to reduce undesirable compounds in several crops including soybean. With regard to the investigations concerning the effects of radiation on storage protein subunits and IVPD, the studies are scarce, and in these studies, gamma rays have been used instead of electron beam as a source of radiation. Compared to gamma rays, EB has less penetrating capacity and requires a high dose rate. Further, these reported studies are limiting in the fact that these have been conducted with only one genotype. However, soybean genotypes have been reported to vary in sensitivity to radiation (Ukai and Yamashita 1980; Mudibu et al. 2012). Afify et al. (2011) studied the effects of gamma rays on subunits of storage protein, namely, β-conglycinin (7S) and glycinin (11S) of soybean seeds of one genotype only. Taghinejad et al. (2009) reported 15.4% increase in IVPD of soybean seeds on exposure to gamma irradiation at a very high dose of 30 kGy. However, the authors investigated only one genotype. Abu-tarboush (1998) studied the effect of gamma irradiation on the IVPD of defatted soy flour, and reported only 5.5% increase in IVPD at 10 kGy dose. The sample exposed to irradiation was fine flour and not the whole soybean seeds. Further, Ebrahimi-Mahmoudabad and Taghinejad-Roubaneh (2011) studied the effect of EB radiation on the content of TI in soybean seeds of one genotype only. In the present investigation, soybean seeds of three genotypes were irradiated with high-speed electron beam at 4.8, 9.2, 15.3 and 21.2 kGy to assess its impact on storage protein viz. β-conglycinin (11S) and glycinin (7S) fraction. Besides, the effects of EB-irradiation on IVPD and trypsin inhibitor content of soybean seeds were also assessed.

Materials and Methods

Samples

Soybean seeds of three genotypes, namely, IC210, NRC107 and JS95–60 were from the crop raised in the fields of ICAR-Indian Institute of Soybean Research. Seed samples packed in the sealed polyethylene bags of size 17 cm × 12 cm and 1.8 mm thickness were exposed to EB-irradiation. Alanine pellets were used as dosimeter, and absorbed doses were measured using an electron paramagnetic resonance system (Bruker, e-scan EPR spectrometer).

Irradiation Set-up

Electron accelerator (10 MeV) LINAC was used for irradiating soybean seeds. Accelerator parameters viz. beam energy, peak current and pulse repetition rate were set at 8.3 MeV, 400 mA and 52 Hz, respectively. Polyethylene bags containing soybean seeds were exposed to EB radiation for 3, 6, 10 and 13 passes, which resulted in the absorbed doses of 4.8, 9.2, 15.3 and 21.2 kGy, respectively. The dose delivered per pass was 1.57 kGy. Alanine electron paramagnetic resonance system was employed to measure absorbed doses. The overall uncertainty in dose measurement was less than 3%.

Densitometry of Storage Proteins

A fixed amount of soluble protein was loaded onto the polyacrylamide gel. The current employed during the movement of the protein in stacking (5%) and running gel (12.5%) was 30 and 70 mA, respectively. After completing the run, the gel was stained with 0.25% coomassie brilliant blue followed by destaining using methanol/water/acetic acid in the ratio of 45:45:10. The protein profile pattern was scanned through densitometer (Bio-Rad G900), and quantification of each protein band was carried out using *Image Lab 5.2.1* software.

In Vitro Protein Digestibility

In vitro protein digestibility (IVPD) of unirradiated and irradiated seed samples were determined by pepsin digestion method as described by Kayembe and Rensburg (2013).

Trypsin Inhibitor Analysis

Trypsin inhibitor (TI) content in soybean seeds was determined by following the standard procedure (Hammerstrand et al. 1981).

Statistical Analysis

All steps and biochemical assays were performed in triplicate samples, and data presented in Table 1 are mean ± standard deviation of the three independent replicates. All the statistical analyses were carried out through *SAS* 14.0, and the comparison between different values was done at 5% significance level.

Results and Discussion

Changes in Glycinin and β-Conglycinin of Storage Protein Subunits

The intensity of glycinin (11S) and β-conglycinin (7S) protein subunits in EB-irradiated seeds were expressed as a

Table 1. Relative density of protein subunits of β-conglycinin (7S) and glycinin (11S) fractions, IVPD and TI content of the EB-irradiated seeds compared to the unirradiated (control) seeds of three soybean genotypes

Genotype	Irradiation dose kGy	β-Conglycinin (7S)			Glycinin (11S)		IVPD (%)	TI (mg/g defatted flour)
		α'	α	β	Acidic	Basic		
IC210	Control	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	38.78 ± 0.02^{a}	71.44 ± 4.5^{a}
	4.8	0.92 ± 0.01^{cd}	0.89 ± 0.00^{de}	0.88 ± 0.01^{de}	0.99 ± 0.03^{bc}	1.03 ± 0.02^{b}	38.19 ± 0.02^{a}	66.63 ± 3.8^{b}
	9.2	0.69 ± 0.01^{h}	0.72 ± 0.02^{gh}	0.80 ± 0.00^{f}	0.82 ± 0.02^{ef}	0.85 ± 0.02^{de}	43.43 ± 0.00^{b}	67.13 ± 3.9^{b}
	15.3	0.58 ± 0.02^{j}	0.49 ± 0.00^{k}	0.75 ± 0.02^{g}	0.89 ± 0.03^{de}	0.94 ± 0.01^{cd}	45.73 ± 0.01^{c}	65.50 ± 5.2^{b}
	21.2	0.45 ± 0.00^{k}	0.38 ± 0.01^{l}	0.67 ± 0.01^{h}	0.76 ± 0.00^{fg}	0.95 ± 0.01^{c}	45.45 ± 0.04^{c}	62.32 ± 5.7^{c}
NRC107	Control	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	43.88 ± 0.06^{a}	90.73 ± 4.2^{a}
	4.8	0.91 ± 0.02^{cd}	0.96 ± 0.01^{bc}	1.0 ± 0.02^{b}	0.97 ± 0.04^{bc}	1.09 ± 0.04^{a}	50.24 ± 0.01^{b}	93.68 ± 4.5^{a}
	9.2	0.83 ± 0.02^{ef}	0.94 ± 0.02^{cd}	0.90 ± 0.02^{d}	0.90 ± 0.02^{d}	0.96 ± 0.04b^{bc}	54.46 ± 0.04^{c}	90.73 ± 4.7^{a}
	15.3	0.85 ± 0.01^{de}	0.94 ± 0.01^{cd}	0.83 ± 0.01^{ef}	0.91 ± 0.02^{cd}	0.97 ± 0.02^{bc}	54.42 ± 0.02^{c}	93.47 ± 5.1^{a}
	21.2	0.74 ± 0.00^{gh}	0.79 ± 0.03^{fg}	0.82 ± 0.01^{ef}	0.83 ± 0.01^{ef}	0.92 ± 0.04^{cd}	53.65 ± 0.03^{c}	87.3 ± 4.0^{a}
JS95–60	Control	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	1.00^{b}	42.57 ± 0.04^{a}	60.26 ± 3.0^{a}
	4.8	0.82 ± 0.00^{ef}	0.81 ± 0.01^{ef}	0.98 ± 0.01^{bc}	0.84 ± 0.04^{e}	1.04 ± 0.04^{b}	51.47 ± 0.01^{b}	61.90 ± 3.2^{a}
	9.2	0.75 ± 0.02^{g}	0.76 ± 0.02^{fg}	0.82 ± 0.04^{ef}	0.82 ± 0.02^{ef}	1.09 ± 0.01^{a}	53.92 ± 0.00^{b}	52.82 ± 3.8^{b}
	15.3	0.65 ± 0.04^{hi}	0.66 ± 0.01^{hi}	0.75 ± 0.04^{g}	0.71 ± 0.00^{h}	0.96 ± 0.03^{bc}	57.28 ± 0.02^{c}	53.82 ± 4.8^{b}
	21.2	0.67 ± 0.01^{h}	0.67 ± 0.01^{h}	0.73 ± ±0.02^{gh}	0.77 ± 0.02^{fg}	1.06 ± 0.01^{a}	57.56 ± 0.02 ^{c}	50.19 ± 3.1^{c}

Values given are mean ± standard deviation of replicated samples. Values of protein subunits of β- conglycinin (7S) and glycinin (11S) fractions across the columns with different superscripts and values of IVPD and TI content with different superscripts in the same column for each genotype are significantly different from each other at $P<0.05$

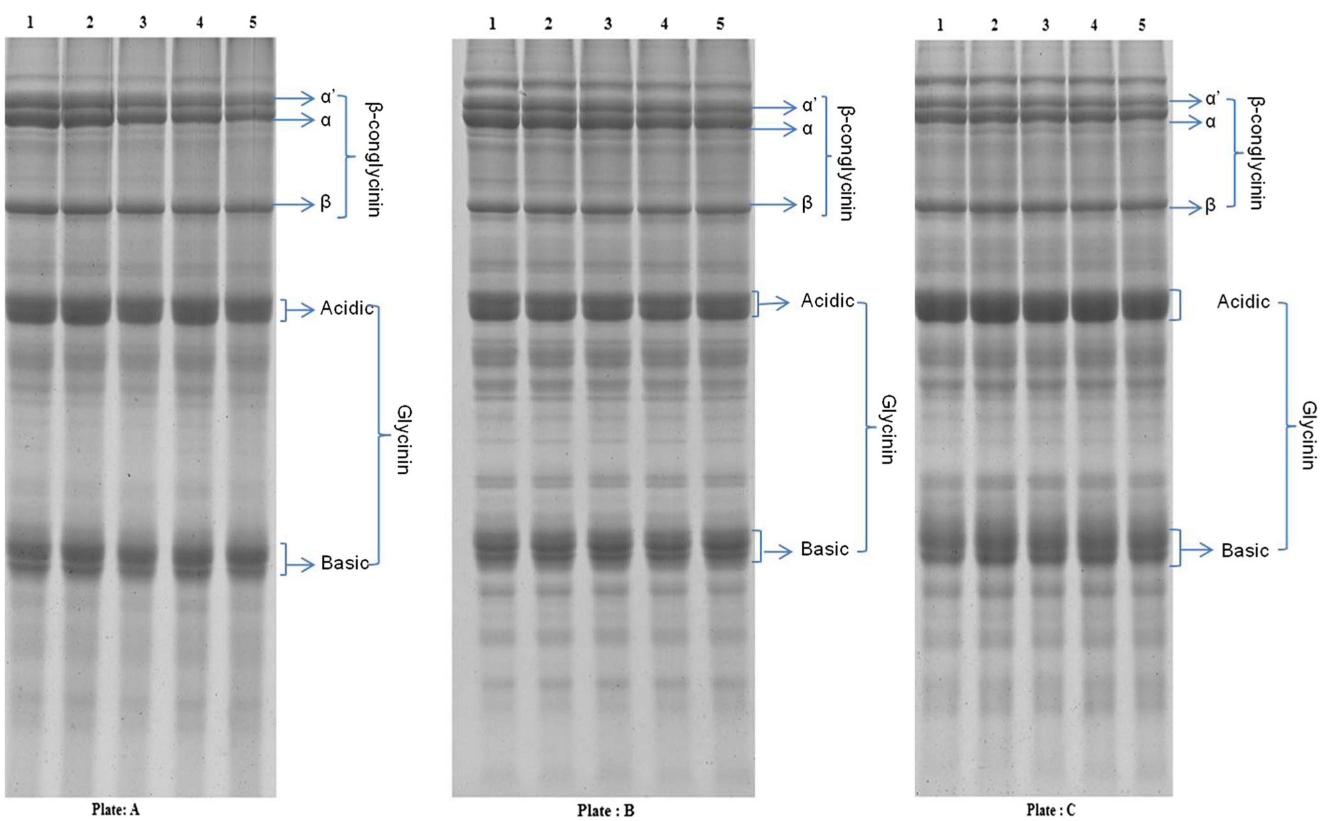

Fig. 1. Plates A, B and C corresponds to storage protein profiling of IC210, NRC107 and JS95–60, respectively. In all three plates A, B and C: lanes 1,2,3,4 and 5 corresponds to irradiation doses of 0 (control), 4.8, 9.2, 15.3 and 21.2 kGy, respectively

relative density in comparison to the concentration of the corresponding subunits in the unirradiated seeds (Table 1). The density of a particular protein subunit in unirradiated seeds was taken as control (1.0). Figure 1 illustrates SDS-PAGE protein profile of the unirradiated and EB-irradiated seeds of three soybean genotypes. With regard to subunits of 7S fraction, in general, significant ($P < 0.05$) decline was observed in the density of α', α and β subunits of β-conglycinin (7S) in three genotypes at all four doses of EB. Genotype IC210 exhibited linear decline in the relative concentration of α', α and β subunits with an increase in dose. Minimum relative density for three subunits of 7S fraction in NRC107 was observed at 21.2 kGy, while in JS95–60 at 15.3 and 21.2 kGy. Across three genotypes and four EB doses, minimum relative density for α', α and β subunits was noted in IC210 at 21.2 kGy.

Glycinin (11S) fraction consists of two major protein subunits, namely, basic and acidic subunit (Fig. 1). As evident from the relative density of the basic subunit at different doses (Table 1), maximum decline for the basic subunit on exposure to EB-irradiation was noted at different doses in three genotypes. Genotypes IC210, NRC107 and JS95–60, showed maximum decline for this subunit at 9.2, 21.2 and 15.3 kGy, respectively. Though, in general, at all doses in three genotypes, basic subunit of 11S fraction exhibited significantly ($P < 0.05$) higher relative density than the acidic subunit but also higher ($P < 0.05$) than α', α and β subunits of 7S fraction. This showed that the basic subunit of 11S fraction degraded least compared to other storage protein subunits. Rather, this subunit registered slight increase ($P < 0.05$) in its density at specific doses of EB-irradiation as noted in JS95–60 and NRC107. With regard to acidic subunit of 11S fraction, this protein subunit showed significantly ($P < 0.05$) higher relative density than α', α and β subunits of 7S fraction in IC210 at all doses. Even in irradiated seeds of the other two genotypes, NRC107 and JS95–60, the relative density of acidic subunit was, in general, significantly ($P < 0.05$) higher than at least two of the three subunits (α', α and β) of 7S fraction. Thus, EB-irradiation caused significantly ($P < 0.05$) less degradation in both basic and acid subunit of 11S fraction compared to α', α and β of 7S fraction. Afify et al. (2011) studied the effect of varying doses of gamma irradiation on the density of protein fractions in soybean. The authors reported an increase in the intensity of the major subunits of 11S-2 (acidic unit of 11S) and 11S-1 (basic subunit) with mol wt. of 38 and 26 kDa, respectively, at 7.5 kGy. In our study, acidic polypeptides did not register an increase in any of the three genotypes at any of the four doses. However, slight increase in the relative density of the basic polypeptides observed at 4.8 kGy in NRC107, and at 9.2 and 21.2 kGy in JS95–60 in our study was in consonance with the results of Afify et al. (2011). The reduction and increase in the intensity of protein fractions on exposure to EB radiation may be because of the degradation or cross-linking of proteins due to the free radicals generated as suggested in an earlier study (Afify and Shousha 1988).

Increase in In Vitro Protein Digestibility

The results given in Table 1 showed genotypic variation for IVPD in soybean genotypes and the changes in this property due to varying doses of EB-irradiation. In general, EB-irradiation resulted in significant ($P < 0.05$) improvement in IVPD in all the three genotypes. The lowest dose (4.8 kGy) caused an increase in IVPD by 14.5 and 20.9% in NRC107 and JS95–60, respectively; though, no significant change was noted in IC210. Further, high doses of EB-irradiation induced significantly higher increase in IVPD compared to low doses. In NRC107, higher dose at 9.2, 15.3 and 21.2 kGy increased IVPD by 24.1, 24.1, 22.4%, respectively, which was significantly ($P < 0.05$) higher than the increase observed in this genotype at 4.8 kGy. In IC210, IVPD increased by 17.9 and 17.2%, which was significantly ($P < 0.05$) higher compared to the increase (12.0%) observed at 9.2 kGy. Similarly, in JS95–60, a significant ($P < 0.05$) higher increase in IVPD was observed at 15.3 and 21.2 kGy compared to EB-irradiation at 4.8 and 9.2 kGy. These observations of EB-induced increase in IVPD are similar to the gamma rays-induced increase (15.43%) in IVPD of soybean seeds at 30 kGy as reported by Taghinejad et al. (2009). Abu-tarboush (1998) reported 5.5% increase in IVPD in the defatted soy flour treated by 10 kGy gamma rays. The increase in IVPD observed in our study is also in consonance with the earlier studies in other crops. Bhat et al. (2008) reported 25.7% increase in IVPD at 15 kGy EB-irradiation in seeds of *Macuna pruriens*. Rousta et al. (2014) reported 10% increase in the protein digestibility of sorghum grains on exposure to 10 kGy EB-irradiation.

Reduction in Trypsin Inhibitor Content

The presence of TI in soybean seeds makes boiling for 20 min a necessary step for the maximum inactivation of this antinutritional factor during processing. Genotypes IC210, NRC107 and JS95–60 exhibited trypsin inhibitor content to the magnitude of 71.4, 90.7 and 60.3 mg/g defatted flour, respectively (Table 1). In IC210, TI content though decreased significantly ($P < 0.05$) at all doses, but maximum decline was only 12.76% at 21.2 kGy. In NRC107, no significant ($P < 0.05$) difference was observed for TI content at all the four doses. In JS95–60, no significant ($P < 0.05$) change in TI content was observed at 4.8 kGy; however, higher doses of 9.2, 15.3 and 21.2 kGy resulted in 12.3, 10.7 and 16.7%, respectively. Ebrahimi-Mahmoudabad and Taghinejad-Roubaneh (2011) reported similar level (19%) of reduction in TI content due to EB dose of 15 kGy. However, higher level of reduction in TI content in soybean seeds due to exposure to gamma irradiation has been reported in literature. de Toledo et al. (2007) reported 28.6% reduction at 4 kGy and 37.6% at 8 kGy in TI content in soybean grains on exposure to gamma irradiation. Dixit et al. (2011) also reported reduction in TI content in soybean to the magnitude of 51.0–63.0% at 5 kGy using gamma radiation. The reduction in TI due to exposure to radiation may be because of the denaturation of protein structure due to the breakage of disulphide bonds, known to be susceptible to radiation exposure.

As evident from Table 1, the reduction in TI due to EB-irradiation was not proportionate to the improvement in IVPD. Genotype NRC107 did not show any change in TI content but still this genotype registered significant ($P < 0.05$) improvement in IVPD at all doses of EB-irradiation. Clemente et al. (2000) also reported similar results in a study pertaining to the investigation of factors affecting IVPD of chick pea albumins. The authors observed that decrease in TI activity was not related to the increase in the rate of IVPD. The increase in IVPD of soybean seeds on exposure to EB-irradiation may be because of denaturation of proteins which may result from conformational changes that can affect nitrogen solubility. Higher decline in glycinin (7S) subunits compared to 11S subunits as a result of EB-irradiation may be because of the fragmentation of larger size of polypeptides constituting 7S fraction. Relatively, smaller size of acidic and basic subunits of 11S fraction compared to α', α and β of 7S fraction makes 11S fraction less vulnerable to fragmentation at the same doses. The increase in intensity of the basic subunit of 11S fraction at specific doses may have resulted from the cross-linking of fragmented polypeptides with larger molecules. Further, all the three subunits of soybean 7S fraction (β-conglycinin) have been reported to be the potential allergens (Krishnan et al. 2009), and a higher ratio of 11S/7S fraction is desirable for better gelling property and hence better quality of soy food products (Kim and Wicker 2005; Poysa et al. 2006). Higher decline in 7S fraction due to EB-irradiation in our study may cause increase in the ratio of 11S to 7S fraction.

Conclusions

In brief, our results showed that EB-irradiation induced higher degradation in β-conglycinin (7S) fraction compared to the glycinin (11S) fraction subunits, and rather, an increase in the density of the basic subunit of 11S fraction was noted at specific doses. This may result in the change in the ratio of 11S/7S fraction, thereby, impacting on the quality and nutritional value of soybean products processed from the irradiated seeds. Therefore, it would be important to assess the yield and the quality of the products like *tofu* processed from the EB-irradiated and non-irradiated seeds of these genotypes in further investigations. The study showed improvement in the IVPD of soybean seeds on exposure to EB-irradiation; however, this increase was not proportionate to the reduction in TI content. In this study, maximum EB dose was 21.2 kGy; whether still higher EB doses can induce higher magnitude of reduction in trypsin inhibitor, and improvement in IVPD in soybean seeds may be investigated in further studies. Further, it would be interesting to study the EB-irradiation induced changes in the conformation of soy protein subunits *vis-a-vis* protein digestibility.

Acknowledgements The study was supported by the Board of Research in Nuclear Sciences, Bhabha Atomic Research Centre, Mumbai (India) under the project bearing Sanction No. 2013/35/49/BRNS.

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