ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 8 (7): 665-670, 2009
ISSN 1682-8356

Effect of Lactic Acid and Irradiation on the Shelf Stability Characteristics of Hurdle Processed Chicken Legs

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Abstract: Hurdle processed chicken legs were subjected for irradiation at 1 and 2 KGy dosage levels after treatment with lactic acid (1 and 2%) to study the effect of irradiation and lactic acid on the shelf stability of the product. The products initially and during storage at 5°C were evaluated for its oxidative rancidity profile in terms of Thiobarbituric Acid Reactive Substances (TBARS), Total carbonyls and non-heme iron values. Lipid oxidation increased with reference to irradiation dosage and there was a significant difference ($p<0.05$) between control, 1 KGy and 2 KGy samples (without lactic acid) after 6 months of storage. Incorporation of lactic acid at 2% levels significantly reduced ($p<0.05$) the TBARS, total carbonyls and non-heme iron values. Hurdle processed chicken legs irradiated at 2KGy with 2% lactic acid exhibited good sensory attributes after 6 months of storage having an overall acceptability score of 7.84±0.31 on a 9 point hedonic scale. The total fatty acid profile by gas chromatography revealed a significant reduction ($p<0.05$) in unsaturated fatty acids in irradiated samples but saturated fatty acids remained unaffected. The values for non irradiated samples during storage did not differ significantly ($p>0.05$). Microbiological profile of the product showed a 3 log reduction in SPC and 2 log reduction in Yeasts and molds by employing 2KGy irradiation dosage and pathogens were also absent.

Key words: Hurdle processing, irradiation, lactic acid, rancidity, chicken, fatty acid, non-heme iron

INTRODUCTION

Extension in shelf-life of perishable foods and maximum retention of desirable initial quality in the processed form of foods are the primary aims of preservation. Since meat is a valuable and perishable commodity, suitable processing and preservation methods have to be selected to meet the needs of the consumer. In our country, Defense services are the largest consumers of processed meat and meat products. Quality alterations have been observed during the existing processing techniques, limiting the shelf-life of the products. Hurdle processing and irradiation are the emerging meat preservation techniques and many of the limitations noticed in the traditional processes can easily be overcome through the use of appropriate hurdles combined intelligently in a sequential manner. More than three decades of research and development work on various technological aspects of food irradiation have clearly demonstrated its predictability and efficacy. So in this study, a multi-hurdle approach including irradiation has been employed, to retain the quality and extend shelf-life of hurdle processed chicken.

Microbial contamination of meat is a serious concern for both meat products and consumers. Radiation processing, has emerged as an alternate technology to eliminate microbial contamination (Kanatt *et al*., 2002). Several countries have approved irradiation of meat and meat products (Molins *et al*., 2001). Wide acceptability of radiation processed meat products will depend upon quality parameters such as oxidative changes, colour stability and organoleptic attributes. Irradiation is known to accelerate lipid peroxidation of meat and meat products (Formanes *et al*., 2003). This is one of the primary causes of deterioration in quality of meat during storage, leading to development of off-flavour, as well as reduced shelf stability and acceptability. It occurs during processing and storage of meat and meat products. Products of lipid peroxidation adversely affect the colour, flavour, texture and nutritive value of meat. So it is necessary to control these changes in irradiated meat products for better product development.

Addition of antioxidants is one of the simplest means of ensuring oxidative stability in irradiated meat. Effect of natural antioxidants like chitosan (Kanatt *et al*., 2004), mint (Kanatt *et al*., 2006) and tocopherol in combination with sesamol (Nam *et al*., 2003) were evaluated on lamb and pork meat during radiation processing and storage to establish the antioxidant potential and found to give positive effects in controlling the oxidation of lipids. There are also reports on the usage of ascorbic acid in meat for the inhibition of lipid oxidation (Verma and Sahoo, 2000). The usage of spices in the hurdle processing and incorporation of lactic acid has been evaluated for effective reduction of peroxidation effect in the irradiated chicken samples. Lactic acid is mainly consumed in food stuffs, where it plays an important role in extending the shelf-life and flavour enhancement (Dutta and Dutta, 2006). In view of this, studies have

been conducted to evaluate the effect of multi-hurdle approach including irradiation on the characteristics of the product during refrigerated storage and to study the effect of irradiation dosage on the chemical and microbiological quality during storage to establish the shelf-life of masala coated chicken legs.

MATERIALS AND METHODS

Sample preparation: Fresh chicken legs (from 6-8 weeks old hens, weighing 150-160 g) were obtained from the local market. Wet masala was prepared using onion, garlic, ginger and spices like cloves cinnamon, cardamom, cumin, chilli powder, turmeric powder, chicken masala, coriander and salt.

The chicken legs after thorough cleaning were marinated in lemon juice for 2 h. After marination, the product was made into 9 equal portions. Out of these 3 portions were treated with 1% lactic acid, the other 3 portions with 2% lactic acid. All these products were cooked and coated with masala. After thorough mixing, it was surface dehydrated at 85-90°C for 1 h, cooled and packed in PFP [paper / foil / polyethylene packages (45 GSM paper / 20 μ Al.foil / 37.5μ low density LDPE)].

Sample codes:

- 0I non irradiated hurdle processed chicken legs,
- 1I 1KGy irradiated hurdle processed chicken legs
- 2I 2 KGy irradiated hurdle processed chicken legs
- 0II non-irradiated hurdle processed chicken legs with 2% lactic acid
- 1II 1 KGy irradiated hurdle processed chicken legs with 2% lactic acid
- 2II 2 KGy irradiated hurdle processed chicken legs with 2% lactic acid.

Irradiation: The samples were subjected to gamma-irradiation at melting ice temperature (1-3°C) in a package irradiator (Nordion Intl. Inc. Ontario, Canada) with a ^{60}Co source, at 1 KGy and 2 KGy dosage levels. The product after irradiation along with control was stored at 5°C for shelf-life evaluation with reference to chemical and microbiological characteristics.

Chemicals: All the chemicals and reagents used in the investigation were of Analar grade obtained from BDH, India. The standard fatty acid methyl esters used in the estimation of fatty acids by gas chromatography and the BF_3-CH_3OH used in the esterification were obtained from Sigma Chemicals Corporation, USA.

Chemical analysis: Proximate composition of the product was determined by the AOAC (1984) procedure. Lipid extraction of the samples was carried out by the method of Folch *et al.* (1957). The products initially and during storage were subjected for chemical evaluation by estimating TBARS (Taraldgis *et al.*, 1960), total carbonyls (Benca and Metchella, 1954) and non-heme Iron (Igene *et al.*, 1985). The catalytic activity of non-heme iron was carried out by first precipitating the bound heme iron with 10 ml of 40% trichloro acetic acid following centrifugation. The supernatant was removed for determination of free non-heme iron by the colorimetric method using 1, 10-phenanthroline as above and calculated the non-heme content from the standard graph.

The sensory characteristics of the Hurdle processed irradiated and non-irradiated chicken legs with lactic acid were evaluated during storage by subjecting these samples to an overall acceptability score on a 9 point hedonic scale by a panel of judges, using the procedure of Murray *et al.* (2001).

Gas chromatography analysis

Esterification of fatty acids: The samples were esterified as per the procedure of Metcalf *et al.* (1966) with slight modifications.

About 150 mg of lipid was accurately weighed into a clean and dry stoppered test tube. 4 ml of 0.5 N alcoholic sodium hydroxide solutions was added and heated for 5 min over a water bath at 90°C. On cooling 5 ml of Boron trifluoride-methanol reagent (14%) was added and heated for 5 min at 90°C over a water bath, followed by addition of 10 ml of saturated sodium chloride solution. The samples were thoroughly cooled to room temperature and 5 ml of hexane was added to each tube. It was shaken well and kept undisturbed. The upper hexane layer was drawn out into clean dry conical flask and dried over anhydrous sodium sulphate to remove the traces of moisture if present. The samples were filtered and transferred to stoppered clean dry tubes for gas chromatographic analysis.

Total fatty acid analysis by Gas chromatography: Analysis of total fatty acids was carried out by ceres-800, Chemito model Gas chromatograph fitted with BPX 70 column (25 m, 0.32 mm ID) and Flame Ionization Detector (FID). Temperature gradient programming was employed from 150-220°C. Split ratio was adjusted to 1:25 and capillary flow of carrier 2 ml/min. Injector and detector port temperatures were adjusted as 230 and 240 respectively. For FID hydrogen and oxygen were used and the flow was adjusted as 45ml/min and 450ml/min respectively. Along with samples standard esters of fatty acids were also injected and the fatty acids were detected by comparing the retention time of the standard esters of fatty acids. The quantification of the fatty acids was carried out by evaluating with the standard fatty acid esters area corresponding to each peak in the chromatogram. Iris -32 software is used to integrate and evaluate the chromatogram in the analysis.

Microbiological analysis: Microbiological characteristics of the product were evaluated by determining SPC, Yeasts and molds, Coliforms and pathogens by standard microbiological procedures (Harrigan, 1998).

Statistical analysis: The data obtained were subjected to analysis of variance (ANOVA) and Duncan's multiple range test to evaluate the statistical significance of the treatments and significance was established at $p<0.05$.

RESULTS AND DISCUSSION

Evaluation of the oxidative deterioration of lipids in terms of TBARS and total carbonyls: The oxidative rancidity parameters in terms of TBARS and total carbonyls for the hurdle processed irradiated chicken samples have been depicted in Fig. 1 and Table 1 respectively. Both hurdle processed and lactic acid (2%) treated hurdle processed chicken samples were subjected to gamma-irradiation at 1 KGy and 2 KGy irradiation dosage levels and stored at 5°C along with control to evaluate the lipid peroxidation characteristics and to study the effect of lactic acid on the lipid oxidation of chicken samples during irradiation and storage. From the data it could be observed that lipid oxidation increased with reference to irradiation dosage and there was a significant difference ($p<0.05$) between control, 1 KGy and 2 KGy processed samples (without lactic acid) after 6 months of storage. Irradiation induced quality changes in meat such as off odour development due to lipid oxidation was earlier reported by Ahn *et al.* (2000). The negative effect of irradiation on the lipid deterioration of meat by producing free radicals was also reported by Thayer *et al.* (1993) and Lakritz *et al.* (1995). Incorporation of lactic acid at 1% level had a moderate effect in inhibiting the rancidity profile during irradiation and storage but lactic acid at 2% level significantly reduced the TBARS and total carbonyl values after 6 months of storage. So the data pertaining to 2% lactic acid are only shown in the results and discussion part. The results clearly indicated the effect of lactic acid in controlling the lipid oxidation connected with irradiation in meat samples. The irradiated product treated with 2% lactic acid was acceptable for more than 6 months and exhibited good shelf stability.

Table 1: Changes in Total carbonyls[a,b] in hurdle processed irradiated chicken legs with Lactic acid.

Sample code	Storage period (months) 0	2	4	6
0 I	0.71±0.08[a]	0.78±0.06[a]	0.90±0.07[a]	1.08±0.07[a]
1 I	0.93±0.02[a]	1.19±0.10[b]	1.31±0.06[b]	1.53±0.11[b]
2 I	1.14±0.07[b]	1.29±0.09[b]	1.73±0.12[b]	2.49±0.13[b]
0 II	0.69±0.05[a]	0.71±0.08[a]	0.84±0.09[a]	0.93±0.09[a]
1 II	0.70±0.03[a]	0.82±0.05[a]	0.89±0.07[a]	1.10±0.05[a]
2 II	0.75±0.04[a]	0.91±0.08[a]	1.05±0.09[b]	1.29±0.08[b]

[a]Values are shown as means ± standard deviation (n = 5).
[b]Within the column, values superscripted with different letters are significantly different($p<0.05$). Values with same letters did not differ significantly ($p>0.05$).

Table 2: Changes in Non heme iron[a,b] in hurdle processed irradiated chicken legs with Lactic acid

Sample code	Storage period (months) 0	2	4	6
0 I	0.42±0.06[a]	0.48±0.02[a]	0.63±0.04[a]	0.72±0.08[a]
1 I	0.49±0.03[a]	0.68±0.05[b]	0.90±0.03[b]	1.12±0.09[b]
2 I	0.59±0.03[a]	0.71±0.06[b]	0.94±0.08[b]	1.58±0.10[b]
0 II	0.39±0.02[a]	0.42±0.02[a]	0.58±0.03[b]	0.63±0.05[a]
1 II	0.41±0.04[a]	0.52±0.06[a]	0.69±0.05[a]	0.79±0.07[a]
2 II	0.50±0.03[a]	0.60±0.08[a]	0.79±0.03[a]	0.89±0.07[a]

[a]Values are shown as means ± standard deviation (n = 5).
[b]Within the column, values superscripted with different letters are significantly different($p<0.05$). Values with same letters did not differ significantly ($p>0.05$).

Evaluation of the catalytic activity of non-heme iron: Transition metals such as iron, copper and cobalt may catalyze the initiation and enhance the propagation steps involved in lipid auto oxidation (Shahidi, 2005). Igene *et al.* (1979) reported the ferrous iron release during cooking and its effect on accelerating lipid oxidation. Non-heme Fe, a free Fe from the heme moiety is usually generated when the meat is cooked and stored. This acts as a catalyst in lipid peroxidation mechanism. Control of the release of non-heme Fe during cooking and storage is expected to help in inhibiting the catalytic activity of Fe and thus lipid oxidation. So the values of non-heme Fe have been monitored during storage of hurdle processed irradiated chicken samples and shown in Table 2. It could be observed that the values were significantly different ($p<0.05$) for control, 1 KGy and 2 KGy hurdle processed (without lactic acid) samples after 6 months of storage. Lipid oxidation associated with non heme iron increase was earlier studied by Jayathilakan, *et al.* (2007) in the case of fluidized bed dried mutton and reported the ability of antioxidants in controlling the catalytic activity of non heme iron. The non-heme Fe values increased with irradiation dosage and storage period. Addition of 2% lactic acid had significantly reduced ($p<0.05$) the values during storage period indicating the effect of lactic acid in controlling the release of non-heme iron and thus lipid oxidation. The results were in positive correlation (r^2 = 0.98) with the data shown in Fig. 1 and Table 1.

Total fatty acid profile by gas liquid chromatography: Fatty acid composition as % of total fatty acids in hurdle processed irradiated chicken samples has been estimated by using Chemito GC, ceres-800 using Iris 32 software. Effect of irradiation dosage on the degradation of fatty acids during storage has been studied and depicted in Table 3. From the values it could be observed that saturated fatty acid like lauric, palmitic and stearic did not show any significant changes ($p>0.05$) in the values because of irradiation and storage at 5°C for

Table 3: Fatty acid composition (as % of total fatty acids)[a,b] of hurdle processed Irradiated Chicken samples

		0 KGy	1 KGy	2 KGy
Lauric	Initial	0.91±0.12[a]	0.83±0.04[a]	0.82±0.04[a]
($C_{12:0}$)	6 m	0.86±0.04[a]	0.79±0.08[a]	0.80±0.05[a]
Palmitic	Initial	21.25±0.4[a]	20.29±0.94[a]	20.06±1.04[a]
($C_{16:0}$)	6 m	20.36±1.04[a]	19.94±0.98[a]	19.46±1.08[a]
Stearic	Initial	18.96±0.98[a]	17.32±1.16[a]	17.11±1.04[a]
($C_{18:0}$)	6 m	17.62±0.89[a]	16.98±0.91[a]	16.53±0.76[a]
Oleic	Initial	36.82±1.49[a]	34.59±1.86[a]	32.18±1.18[b]
($C_{18:1}$)	6 m	35.09±1.89[a]	32.24±1.35[b]	29.49±0.91[c]
Linoleic	Initial	13.05±0.56[a]	12.00±0.72[a]	11.14±0.59[a]
($C_{18:2}$)	6 m	12.16±0.48[a]	10.18±0.61[b]	8.19±0.62[c]
Linolenic	Initial	3.36±0.39[a]	3.04±0.41[a]	2.52±0.36[a]
($C_{18:3}$)	6 m	2.89±0.19[a]	2.01±0.26[b]	1.43±0.41[c]
Arachidonic	Initial	1.85±0.11[a]	1.68±0.10[a]	1.33±0.12[b]
($C_{20:4}$)	6 m	1.36±0.09[a]	1.08±0.05[b]	0.79±0.03[c]

[a]Values are shown as mean ± S.D (n = 5). [b]Within the rows values superscripted with different letters are significantly different($p< 0.05$). Values with same letters did not differ significantly ($p>0.05$)

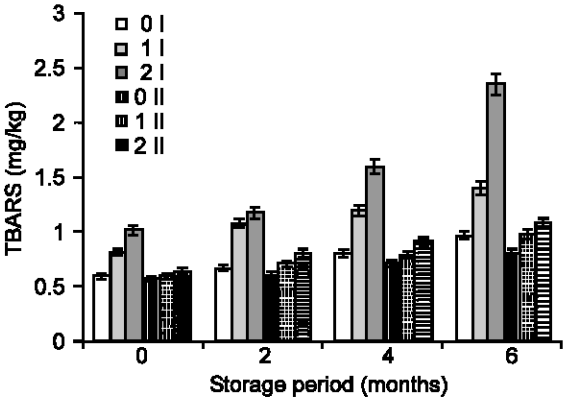

Fig. 1: Changes in TBARS in hurdle processed irradiated chicken legs with Lactic acid

6 months. But the unsaturated fatty acids showed significant difference ($p<0.05$) after 6 months of storage with initial values. The values for oleic, linoleic, linolenic and arachidonic showed significant variation after irradiation and storage. Non irradiated samples during storage did not differ significantly ($p>0.05$). Oxidation of lipids is one of the primary causes of deterioration in meat systems during cooking and storage, leading to development of off flavour, decrease in nutritive value, loss of colour and texture etc (Morrissey *et al*., 1998). The substrate for the lipid oxidation reaction is mainly unsaturated fatty acids (Simic and Taylor, 1987). So the degradation of unsaturated fatty acids present in hurdle processed chicken samples with reference to different dosages of irradiation was investigated. So from the data obtained it could be observed that irradiation and storage of these samples did have an impact on the concentration of unsaturated fatty acids present in the samples.

Microbiological analysis: Microbiological profile of hurdle processed irradiated chicken samples in terms of SPC, Coliforms, *Staphylococcus aureus* and Yeasts and Molds has been evaluated during storage and shown in Table 4. It could be observed from the table that the reduction in SPC and Yeasts and molds does have a direct impact on the irradiation dosage employed in the study. By employing 2 KGy dosage, a 3 log reduction in SPC and 2 log reduction in Yeasts and molds could be achieved. The shelf life extension by treating with low doses of irradiation for the control of microbial spoilage was studied earlier by Paul *et al*. (1990). There are several reports on the radiation processing of meat products like bacon, ham (Weirbicki and Heilgman, 1980) sausages (Kiss *et al*., 1990) and beef burgers (Dempster *et al*., 1985). In addition to spoilage bacteria, meat product may contain parasites and pathogenic bacteria, which could be eliminated by irradiation. The radiation doses required to inactivate 90% of the colony forming unit of the common food borne pathogens associated with meat and meat products are in the range of 1-4 KGy (Thayer and Bond, 1992). Coliforms and *Staphylococcus aureus* were nil initially in the samples because of the safety protocol followed during hurdle processing. Lactic acid incorporation did not make any significant changes in the microbial profile during storage. So 2 KGy irradiation coupled with hurdle processing could extend the shelf-life to more than 6 months in terms of microbial safety of the product.

Proximate composition and organoleptic characteristics: Table 5 illustrates the proximate composition of the Hurdle processed irradiated chicken legs prepared with lactic acid. The moisture was found to be 39.18%, in this intermediate moisture ready-to-eat product. The protein content was 37.35%, fat 16.83% and total ash was found to be 4.52%. The sample had a moderate percentage of fat, considerable amount of protein and a calorific value of 305 Kcal/100g.

Organoleptic characteristics of the Hurdle processed irradiated and non-irradiated chicken legs with 2% lactic acid were evaluated during storage for 6 months at 5°C and the overall acceptability score is shown in Table 6. The score was calculated on the basis of evaluating the texture, taste, flavour, chewability, colour and other related aspects on a 9 point hedonic scale. Initially and upto 2 months, all the samples had good overall acceptability scores, while after 3 months of storage, samples 0I and 0II (non-irradiated samples) exhibited significant reduction ($p<0.05$) in organoleptic score as compared to other samples. After 6 months of storage the samples irradiated at 1 and 2 KGy dosages with lactic acid exhibited good sensory attributes as indicated in the organoleptic score of 7.1 and 7.84 respectively. All the other samples showed a significant reduction ($p<0.05$) in the overall acceptability score as reflected in the values in Table 6. These values positively correlated with other chemical and microbiological parameters.

Table 4: Microbiological profile of hurdle processed, irradiated chicken sample

	SPC (cfu/g)				Yeasts and Molds (cfu/g)			
	Storage period (months)							
Sample code	0	2	4	6	0	2	4	6
0 I	3.7×10^4	3.8×10^4	2.9×10^5	3.9×10^5	2×10^2	1.8×10^2	3.2×10^2	2.9×10^2
1 I	5.8×10^2	7.3×10^2	1.9×10^3	5.4×10^3	1.8×10^1	2.2×10^1	1.9×10^1	1.6×10^1
2 I	2.5×10^1	2.5×10^1	3.5×10^2	8.5×10^2	<10	<10	<10	<10
0 II	2.9×10^4	3.1×10^4	2.7×10^5	3.5×10^5	1.7×10^2	1.6×10^2	2.5×10^2	2.4×10^2
1 II	3.9×10^2	6.1×10^2	1.3×10^3	4.9×10^3	1.5×10^1	1.7×10^1	1.5×10^1	1.3×10^1
2 II	1.8×10^1	2.2×10^1	2.8×10^2	7.8×10^2	<10	<10	<10	<10

Coliforms and *S.aureus* were nil initially and throughout the storage period. Values are shown as mean ± S.D (n = 5).

Table 5: Proximate composition[a] of Hurdle processed irradiated chicken legs

Parameter	% composition
Moisture	39.81 ± 0.93
Protein	37.35 ± 1.12
Fat	16.83 ± 0.76
Total Ash	4.52 ± 0.36

[a]values are shown as means ± standard deviation (n = 5)

Table 6: Organoleptic characteristics of Hurdle processed irradiated chicken legs with lactic acid, expressed as overall acceptability score[(a,b)] during storage at 5°C

	Storage period (months)			
Sample code	0	2	4	6
0 I	8.25±0.31[a]	7.91±0.22[a]	6.08±0.24[b]	5.18±0.19[b]
1 I	8.18±0.23[a]	8.01±0.24[a]	6.93±0.16[a]	6.24±0.30[b]
2 I	8.04±0.15[a]	7.93±0.23[a]	6.63±0.31[a]	6.1±0.12[b]
0 II	8.31±0.16[a]	8.04±0.22[a]	6.31±0.21[b]	5.6±0.18[b]
1 II	8.11±0.30[a]	8.04±0.13[a]	7.49±0.22[a]	7.1±0.30[a]
2 II	8.30±0.32[a]	8.11±0.24[a]	7.94±0.16[a]	7.84±0.31[a]

[a]Values are shown as means ± standard deviation (n = 10).
[b]Within the column, values superscripted with different letters are significantly different($p<0.05$). Values with same letters did not differ significantly ($p>0.05$)

Conclusion: Hurdle processing and irradiation are the emerging food preservation techniques and they could be successfully employed in the development of meat products. Hurdle processed chicken samples exhibited 3-4 months shelf-life in terms of chemical and microbiological parameters. Irradiation of the hurdle processed chicken samples at 2 KGy with 2% lactic acid could extend the shelf-life to 6-7 months at 5°C. The lipid peroxidation during irradiation and storage could be successfully controlled by addition of 2% lactic acid. Irradiated samples during storage showed significant degradation in unsaturated fatty acids i.e., oleic, linoleic, linolenic and arachidonic. The 2KGy irradiated sample with 2% lactic acid exhibited a good organoleptic score of 7.84±0.31 after 6 months of storage indicating good shelf stability aspects. By adopting hurdle processing and irradiation we could achieve a microbiologically safe product. On the whole, hurdle processing coupled with irradiation is a promising technique in developing shelf stable meat products benefiting both civilian and service sectors.

ACKNOWLEDGEMENT

The authors wish to express their deep sense of gratitude to Dr. A.K. Sharma, Dr. Ramesh Chander and their team of scientists in the Food Technology Division, BARC, Mumbai for rendering help in executing the irradiation of the products.

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