International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.6, pp 1197-1205, 2017

Microbial analysis of electron beam irradiated *Capsicum annum*

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Abstract : Food irradiation is the process of exposing food to ionizing radiation to destroy microorganism's bacteria, viruses or insects that might be present in the food.

Radiation processing of food involves exposure of food to short wave radiations energy to destroy the contaminants (bacteria, fungi etc.,) and thereby to achieve extension of shelf life. Since these electromagnetic radiations (electron beam, x – rays) are highly penetrating and no residual radiation retained in the materials. Irradiation treatment is considered as more safe and effective than chemical method.

Key words : *Capsicum annum,* electron beam irradiation, KGy, microbial load.

1. Introduction

Chilli is considered as one of the commercial spice crops. In daily life chillies are integral and the most important ingredient in many different cuisines around the world.

India is the world leader in chilli production followed by china and Pakistan. Indian chilli is considered to be world famous for two important commercial qualities. Its colour and pungency levels. Indian share in global production is 50 to 60 percentage. However India is the only source for hot chillies. Chilli is cultivated in tropical and sub – tropical climates mostly as a rain fed crop in India. Chilli is a seasonal and annually grown cash crop. Dried chillies are rich in vitamin A and D. chillies are excellent source of Vitamins A, B, C and E with minerals like Molybdenum, Manganese, foliate, Potassium, thiamine and copper.

In India chillies have been include in Ayurveda medicine and used as tonic toward – off many diseases. Chilli is good for slimming down as it burns the calories easily. It stimulates the appetite, helps to clear lungs and stimulate the digestive systems. It is also used in beverages. The extractedcapsaicin is used in the apin balms, cosmetics and pharmaceutical industry. Capsaicinaa pigment derived from chilli is used for natural colouration in jam and jelly preparation.

The quality of dried chilli is assessed by a number of different parameters such as colour, hotness, ascorbic acid content and volatile flavour compounds [1-3]. Major chilli growing countries are India, china, Pakistan, Indonesia, Korea, Turkey and Srilanka. Food irradiation is processing of food products by ionizing radiation and it is one of the preservation method to control the food borne pathogens. Reduction of microbial load, sprout inhibition also happens. Insect manifestation, preserve the nutrional values and extend the shelf life of period. The quality evaluation of food product is useful to improve the processing condition for getting the better quality products. Therefore the aim of this present study was using the electron beam irradiation to preserve quality of the red chilli and to analyse the microbial load of red chilli after irradiation.

Principle of electron beam irradiation

Low or high energy electron beam radiation generated by electron accelerator (usually 10 MeV) can damage DNA in living cells directly or get – H, - OH from radiolysis of water or small molecules directly by high energy pulse. Living radical with nuclear material can initiate cross link [4] .Thus we can kill pests, post-pone fruit and vegetable ripening and senescence and also extend shelf life of the farm products.

Irradiation can be used to decontaminate or reduce microbial burden in food and medicinal products [5].Irradiation process improves the shelf life of fruits, vegetables and spices by destructing the micro flora and provides a suitable alternative to chemical treatment [6]

Physical methods

The physical methods measure the effects of the radiation generated radicals or trapped electrons in the solids. These methods may either leave free radicals and electrons unchanged or stimulate some of the electrons and measure their absorbed radiation energy, they are practically involved with their radiation generated defects by dissolution of the solid substances[7]

Food preservation

Food is a perishable commodity. The primary objective of food preservation is to prevent or slow down the growth of micro-organisms including mould, yeast and bacteria as the growth of these micro – organisms causes spoilage of food.

Scope of food preservation

To increase the shelf life of food as well as its supply. Although the freshness, palatability and nutritive value may be altered with time delay, perishable foods can be preserved to prevent spoilage and made to be available throughout the year. To save food for future use at the time of scarcity or drought etc., after suitable preservation and proper storage. Preservation of food also minimises the preparation time and energy at home.

Spices

Spices are highly valued products and provide a major part of foreign exchange for producer countries. Most spices are rich in antioxidants and anti-microbial, and as a result, their use as natural preservatives in food industries has increased. Common spices include: black pepper, cardamom, cumin, fenugreek, saffron, garlic, fennel, turmeric, ginger and chilli. The moisture in spices is usually low, which largely protects them against microbial spoilage.

Inadequate drying and storage are primarily responsible for fungal contamination. Drying in most the producer countries is primarily by spreading them on floors. Spices harbour some microbes capable of causing spoilage or diseases [8] The most commonly isolated toxigenic fungi include Aspergillus flavus and others belonging to Alternaria, Penicillium, Fusarium and Mucor. Currently, chemical fumigants [9] (e.g. ethylene oxide) and thermal treatment with steam are most commonly used to disinfect spices. Fumigants are less effective and traces of harmful toxic residues may be retained, while thermal treatment may alter the aroma and flavor of spices. Stringent quality specification has been introduced in some of the leading importing countries like US, Germany UK, Netherlands, France and Japan to ensure high quality and safety.

The roles played by FDA, American Spice Trade Association (ASTA) in USA and provision under the Law on Food and Consumer Goods (LFCG) (August 1974) in Germany is worth referring to. Based on these regulations, if the defect level exceeds permissible limits, legal action can be enforced or the exporting country can be blacklisted (e.g. toxin limits: aflatoxins, 4 ppb; AFB1, 2 ppb). Processing spices by gamma irradiation (10-14 kGy) has been well established and is an approved technology to improve the quality in many European countries, US and India.

Gamma irradiation has been successfully implemented and studied for quality amelioration of spices [10]. Gamma-irradiation for microbial decontamination of whole spices and spice powders is legally permitted in some 34 countries worldwide [11] and 23 countries are using this technology commercially [12]. Several studies on spices treated with doses up to 10-15 kGy have shown that no substantial changes occur in volatile oils, flavour profiles, spicing power [8], and antioxidant properties [13]. However, information on colour power and its stability during storage of irradiated spices is scanty. The present study was, therefore, aimed to examine the effect of irradiation on the colour power and its stability during ambient temperature storage of turmeric and red chilli powders which are important commercial sources for natural colourants [14]. Improving the quality of spices without impinging on the original quality and flavor is a critical task and thus the application of EBI can play a significant role in preserving and enhancing the commercial value of spices and their blends.

Effect of irradiation on microorganisms

A large amount of data is available on the sensitivity of microorganisms to irradiation processing; this varies greatly from microorganisms to microorganism and is also dependent upon other extrinsic factors. Vegetative cells are less resistant to irradiation than spores, whereas moulds have a susceptibility to irradiation similar to that of a vegetative cell. However some fungi can be as resistant as bacterial spores[15] Compared to bacteria, viruses generally require higher radiation doses for inactivation [16]. Microbes associated with fresh-cut fruit and vegetable products can vary greatly in accordance with the product type and storage conditions [17]

Dose affects the effect of preservation directly when radiation dosage was added to 0.1 – 1 KGy, it showed the inhibition of microbial growth and reproduction [18], found that if the dosage is too low, it can only inhibit after ripening of farm products and it cannot inhibit the growth of microbes. Researchers also show that radiation dosage was too high to be harmful to form products [19] Found that if the record of peaches storage was about 0.2 KGy can extend their shelf life. But when the dosage was high to 0.5KGy, it can be harmful to the peaches.

2. Materials and methods

2.1 Processesing and packaging:

The Red chilli (*Capsicum annum*) fruits of three different kinds namely Naadu Natural (NN), Naadu Natural Pesticide (NNP) and Naadu Natural Mundu (NNM) were procured from Virudhunagar District, Tamilnadu, India. Virudhunagar is famous for Red chilli production. Before exposing the Red chilli samples to irradiation the fruit samples was cleaned to remove dust particles. The Red Chilli samples were segregated and cut in to small pieces and were packed in PP (Poly Propylene) bags with a dimesnsion of 4 X 4 Cm size.

Table 1 Some Examples Of Current Uses Of Food Irradiation In Different Countries

Region	Country	Food	Maximum Dose (KGy)
America	USA	pork	1.0
		poultry	3.0
	Canada	potatoes	0.1
		spices	1.0
	Brazil	Strawberries	3.0
		Fish	2.2
EUROPE	France	Egg white	4.0
		Cheese	3.5
	Netherland	Dried fruits	1.0
		Frog leg	5.0
	U.K	Roots	0.2
		Shellfish	3.0
ASIA	China	Garlic	0.1
	Thailand	Rice	1.0
		Mangoes	1.0
	South Africa	Fruit juice	3.0

Source: Farkas 1988: Kilcast 1995

2.2 Irradiation dosage:

Food absorbs and is heated up by radiant energies. Radiant energies can kill microorganisms without marked increase of temperature as well as marked changes in the nature of food.

Gamma rays, X – rays and electromagnetic, UV radiations are commonly used for food preservation. Irradiation can be used in a wide range of areas in food preservation.

a) Poultry products

b) Fruits

c) Prevention of sprouting in potatoes and onions

d) Delaying ripening in fruits

e) Preservation of sea foods

f) Prevention of insects' infestation in dry foods and foods products.

The Red chilli (*Capsicum annum*) fruits of three different kinds namely Naadu Natural (NN), Naadu Natural Pesticide (NNP) and Naadu Natural Mundu (NNM) were exposed to Electron beam irradiation of varying dosages say 2, 4, 6 & 8 KGy at room temperature. Irradiation was carried out by exposing both sides for uniformity, non irradiated Red chilli serves as Blank.

2.3 Microbiological studies

Standard plate count method was employed to enumerate the total microbial load in terms of colony forming units in the blank and irradiated samples after 90 days. The samples were serially diluted and plated separately on rose Bengal agar, salmonella, eosin methylene blue agar for enumeration of fungi, salmonella and e.coli respectively.

A 2.5 g of Red chilli samples were weighed, rinsed in a 25 mL beaker containing 10 mL of sterile distilled water and rinsed water samples were diluted 1:10-2 and 1:10-4 . 0.1 mL of each dilution was spread on Mac Conkey agar (Himedia, India) and the plates were incubated at 37 °C for 24 h for isolation of bacteria (Khan et al., 1992). Investigation Total viable counts were determined by counting both red and non-red colonies growing on the plates. Based on their morphological characteristics, red and non-red colonies were selected from each Mac Conkey agar plate for Gram staining, biochemical tests namely: IMViC (Indole, Methyl Red, Voges-Proskauer, citrate), urease, oxidase, catalase, triple sugar iron; and sub-culture on differential media (eosin methylene blue agar at 37 °C and 44 °C for 24–48 h; and mannitol salt agar, and cetri mannitol salt agar, and cetrimide agar at 37 °C), nutrient agar [20]).

3. Results and discussion

It is clear from the above results that all the blank samples of three varieties namely Naadu natural (NN), Naadu natural pesticide (NNP) and Naadu natural Mundu (NNM) were found to contain Total viable count (TVC), E.Coli and fungi to a maximum when compared to the irradiated samples. When we compare the Total viable count, E.Coli and Fungi values of Irradiated samples of Naadu Natural Pesticide and Naadu Natural Mundu the Fungi and E.Coli was found to be absent the value of Total viable count was found to decrease with the increase in dosage. Whereas in the case of Naadu Natural the value of E.Coli and Fungi were found to be absent and unexpectedly the total viable count was found to increase with the increase in dosage. Thus it is advisable to irradiate the Naadu natural pesticide variety and Naadu natural Mundu variety to get a better prevention from microbes.

Table 2 : Food permitted for irradiation under FDA's regulations

S.No	Food	Purpose	Dose
1	Fresh food	Growth and maturation inhibition	1.0
2	Dry spice	Microbial disinfection	30.0
3	Poultry	Pathogen control	3.0
4	Frozen meat	Sterilization	44.0
5	Refrigerated meat	Pathogen control	4.5
6	Syhell eggs	Pathogen control	3.0
7	Seeds for sprouting	Pathogen control	8.0

Table 3a. Microbial load of irradiated & non irradiated Naadu Natural Pesticide Red chilli samples

Parameter	**Blank**	**2KGy**	**4Kgy**	**6KGy**	**8KGy**
TVC CFUs/g	**12,000**	**700**	**628**	**412**	**92**
E.Coli CFUs/g	**12**	**Nil**	**Nil**	**Nil**	**Nil**
Salmonella sp. CFUs/g	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
Fungi CFUs/g	**30**	**Nil**	**Nil**	**Nil**	**Nil**

Table 3b Microbial load of irradiated and non irradiated Naadu Natural Red chilli samples.

Parameter	**Blank**	**2KGy**	**4Kgy**	**6KGy**	**8KGy**
TVC CFUs/g	**14,500**	**100**	**120**	**141**	**184**
E.Coli CFUs/g	**18**	**Nil**	**Nil**	**Nil**	**Nil**
Salmonella sp. CFUs/g	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
Fungi CFUs/g	**30**	**Nil**	**Nil**	**Nil**	**Nil**

Table 3c Microbial load of irradiated and non irradiated Naadu Natural Mundu Red chilli samples

Parameter	**Blank**	**2KGy**	**4Kgy**	**6KGy**	**8KGy**
TVC CFUs/g	**45,700**	**2700**	**1812**	**180**	**220**
E.Coli CFUs/g	**21**	**Nil**	**Nil**	**Nil**	**Nil**
Salmonella sp. CFUs/g	**Nil**	**Nil**	**Nil**	**Nil**	**Nil**
Fungi CFUs/g	**40**	**Nil**	**Nil**	**Nil**	**Nil**

Table 4a. Annova method of One way analysis of comparing Blank with 2KGy

	2KGy with blank		**Total**
N	3	3	6
ƐX	3410	72200	75610
Mean	1136.67	24066.67	12601.67
$ƐX^2$	7780100	2442740000	2450520100
Std.Dev	1397.16	18776.67	17307.27

Table 4b. Annova method of One way analysis of comparing Blank with 4KGy

	4KGy with blank		**Total**
N	3	72200	6
ƐX	2560	24066.67	74760
Mean	853.333	2442740000	12460
$ƐX^2$	3692128	18776.67	2446432128
Std.Dev	868.215	72200	17406.45

Table 4c. Annova method of One way analysis of comparing Blank with 6KGy

	6KGy with blank		Total
N	3	3	6
ƐX	733	72200	72933
Mean	244.33	24066.67	12155.5
$ƐX^2$	222025	2442740000	2442962025
Std.Dev	146.507	18776.67	17643.27

Table 4d. Annova method of One way analysis of comparing Blank with 8KGy

	8KGy with blank		Total
N	3	3	6
ƐX	496	72200	72696
Mean	165.333	24066.67	12116
$ƐX^2$	90720	2442740000	2442830720
Std.Dev	66.01	18776.67	17675.10

Table 5a. Result details of Annova method of comparing Blank with 2KGy

SOURCE	**SS**	**DF**	**MS**	
Between	788677350	1	788677350	
Within	709030733.33	4	177257683.33	F= 4.44933
Total	1497708083.33	5		

Table 5b. Result details of Annova method of comparing Blank with 4KGy

SOURCE	**SS**	**DF**	**MS**	
Between	808288266.67	1	808288266.67	
Within	706634261.33	4	176658565.33	F= 4.57543
Total	1514922528	5		

Table 5c. Result details of Annova method of comparing Blank with 6KGy

SOURCE	**SS**	**DF**	**MS**	
Between	851255349.16	1	851255348.17	
Within	705169595.33	4	176292398.83	F= 4.82866
Total	1556424943.5	5		

Table 5d. Result details of Annova method of comparing Blank with 8KGy

SOURCE	**SS**	**DF**	**MS**	
Between	856910602.67	1	856910602.67	
Within	705135381.33	4	176283845.33	F= 4.86097
total	1562045984	5		

4. Statistical analysis

Mean and standard deviation (SD) of the samples were determined using the statistical package of the samples were (SPSS) version 12 software package. The one-way analysis of variance (ANOVA) is used to determine whether there are any statistically significant differences. Table 4a, b, c and d shows Annova method of One way analysis of comparing Blank with 2KGy, 4KGy, 6KGy and 8KGy. Whereas the Table 5a, 5b, 5c and 5d shows the result details of Annova method of comparing Blank with 2KGy, 4KGy, 6KGy and 8KGy Significance was accepted at 5% probability level. All the data reported in the tables are average values of the one way analysis using annova method.

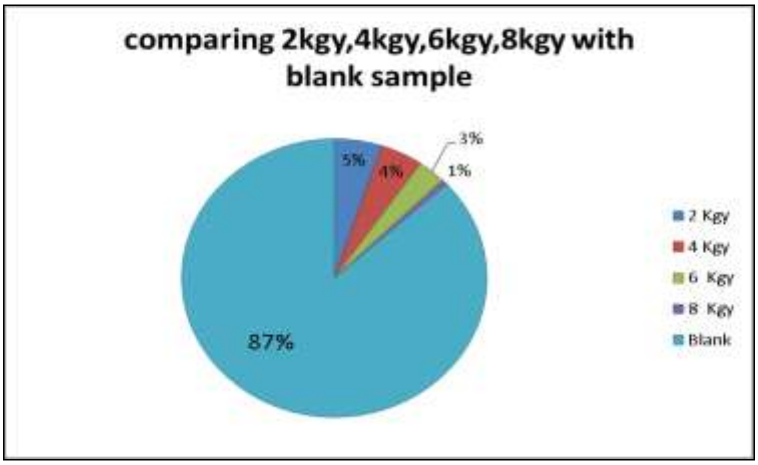

Fig.1 A comparative pie chart showing the total viable count in Blank and Irradiated samples of various dosages (2, 4, 6, & 8 kGy)

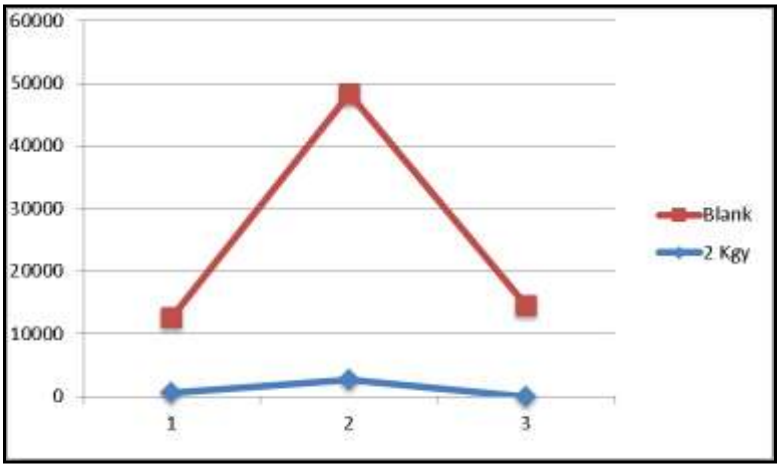

Fig.2 A comparative graph showing the value of Blank with 2KGy irradiated sample of Naadu Pesticide, Naadu Natural Mundu and Naadu Natural

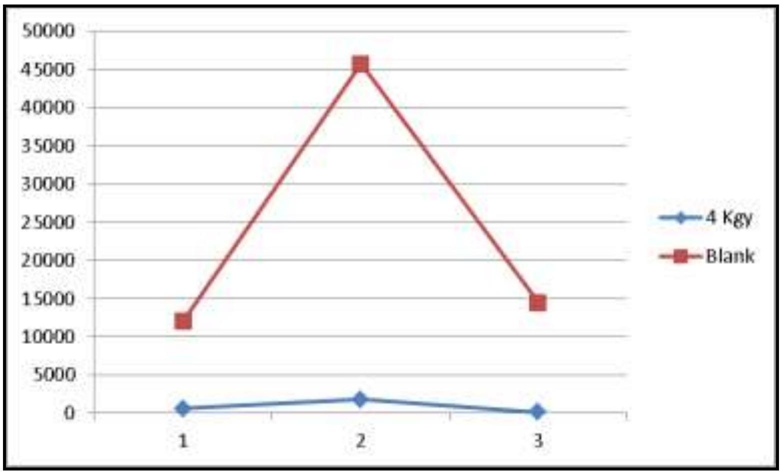

Fig. 3 A comparative graph showing the value of Blank with 4KGy irradiated sample of Naadu Pesticide, Naadu Natural Mundu and Naadu Natural

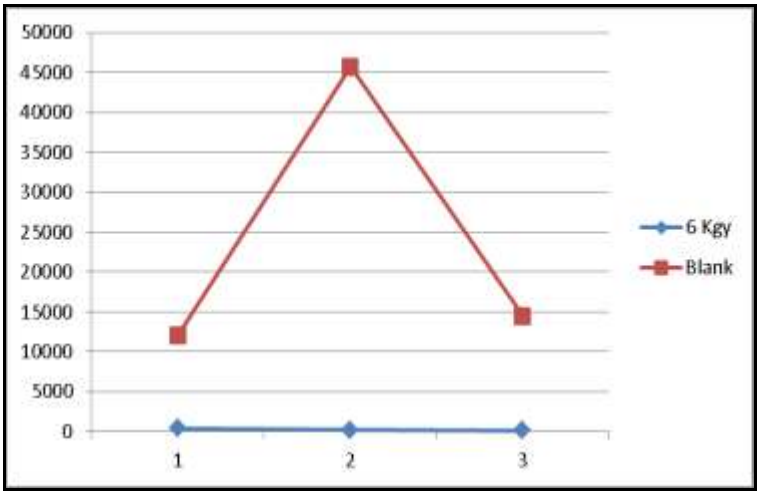

Fig. 4 A comparative graph showing the value of Blank with 6 KGy irradiated sample of Naadu Pesticide, Naadu Natural Mundu and Naadu Natural

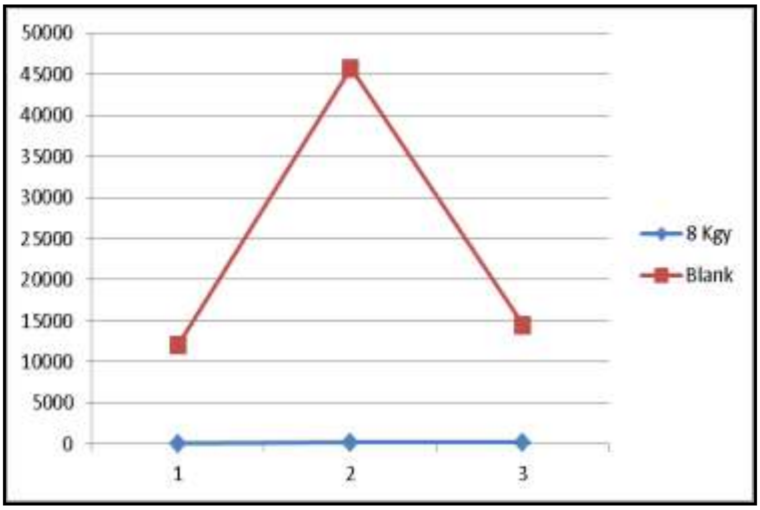

Fig. 5 A comparative graph showing the value of Blank with 8KGy irradiated sample of Naadu Pesticide, Naadu Natural Mundu and Naadu Natural

5. Conclusion

Electron beam irradiation preservation techniques is efficient, safe, non polluting, no residue and keeping the electrons. In the present study electron beam radiation did not cause any significant change, a irradiated red chilli sample. This study shows the efficiency of electron beam irradiation as a processing technology for maintaining the overall quality of irradiated samples.

Acknowledgement

Authors are grateful to DAE – BRNS, Mumbai, India for the financial support (No: 34(1)/14/33/2014 – BRNS and as Dr.Ganesh Sanjeev and Faculties of Microtran Centre, Mangalore University, Karnataka, India for their help to irradiate the samples.

Conflict of interest

The authors declare no conflicts of interest.

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