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Standardization of gamma irradiation dose for selected microbial decontamination in red chilli samples of two different varieties

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Abstract
Red chilli (*Capsicum annuum*) is belonged to the Solanaceae family and it is one of the most common, important spice cultivated and consumed all over the India. It is also known with different names such as chillies, chile, hot peppers, bell peppers, red peppers, pod peppers, cayenne peppers, paprika, pimento and capsicum in different parts of the world. Microbial decontamination is one of the major concerns worldwide due to a) lower shelf life and b) production of mycotoxin during storage period of the spice. The experiments were designed and conducted with the broad aim of reducing microbial load of dried red chilli of two different varieties using gamma irradiation treatment. Decimal reduction dose D_{10} values (radiation resistance) of the potentially pathogenic microorganisms: *Aspergillus flavus, Aspergillus niger, Rhizopus fusarium* sp isolated from chilli samples were 1.2, 1.1, 0.9 and 1.1 kGy, respectively. Complete decontamination was observed at the radiation dose of 7.5 kGy in the both varieties and forms of the dried chilli samples having different moisture content (8 to 14%).

Keywords: Fungus, red chilli, haemocytometer, gamma irradiation

Introduction
Spices are well-known as appetizers and are considered volatile in the culinary art all over the world. Spices are an important group of agricultural commodities being used to aid flavor, colour, taste and nutritional values in the food (Singh, *et al.* 2004) [19]. Red pepper is often contaminated with high levels of moulds, yeasts and bacteria during harvesting, handling, transportation and storage. Utilization of heavily contaminated spices in food process can accelerate the putrefaction of manufactured food products, reduce food shelf-life, and even impair consumers' health. Fungal and bacterial pathogens like *A. flavus, Rhizopus, Helminthosporium, Alternaria alternate, Colletortichum capsici, Fusarium Clostridium perfringens, Staphylococcus aureus*, and *Bacillus cereus*, and high levels of aflatoxin were found in powdered red pepper (Oularbiand Mansouri, 1996, Banerjee and Sarkar, 2003, Bucken huskes and Rendlen, 2004, Aydin, *et al.* 2007) [16, 3, 4, 2]. Therefore, decontamination of spices that are used as an ingredient in processed foods is necessary in order to reduce the number of spoilage microorganisms and eliminate pathogens.
Conventional decontamination methods i.e. heating and fumigation etc. are not suitable for spices treatment. heat cannot be used due to the thermo ability of many essential oil components, while ultraviolet radiation is not effective in decontaminating great volumes due to its short penetration (Fine and Gervais, 2005) [8]. Fumigation with ethylene and propylene oxide are being progressively banned in several countries because of their toxic, mutagenic and carcinogenic effects (Fowles, *et al.* 2001) [9].
Gamma irradiation is recognized as a technically feasible method for reducing post-harvest food losses, ensuring the hygienic quality of food and preservation of food, extending its shelf life and facilitating wider food trade (Farkas, 1998, Lee, *et al.* 2005, Maity, *et al.* 2009, Farkas and Mohacsi-Farkas, 2011) [5, 11, 12, 6]. The chemical structure of irradiated food is less modified than heat-treated one and this technique avoids the use of potentially harmful chemicals (Siddhuraju, *et al.* 2002) [18]. Irradiation treatments have been used to reduce the microbiological load of dehydrated paprika. Irradiation doses up to 12.5 kGy showed no significant differences between the colour properties of irradiated and non-irradiated samples

(Nieto-Sandoval, *et al.* 2000) [15]. However, another study suggested that higher irradiation doses and a longer storage period, resulted in a significant (p<0.01) reduction of all the carotenoids, except capsorubin (Topuz and Ozdemir, 2003) [20]. Higher irradiation doses (above 10 kGy), the structural properties of fibrous carbohydrates can be degraded, and lipids can become somewhat rancid, leading to a loss of food quality. The irradiation of lipids at high doses in presence of oxygen, can lead to the formation of lipid hydroperoxides (Miller, 2005) [14].

Materials and Methods

Dried Red Chilli

For this investigation, a bulk of samples of 5 kg each of different varieties of dried red chilli, namely Kashmiri (known for dark red colour) and Sannam 4 (known for higher pungency) were procured from Gopal masala works, Sardargunj, Anand, Gujarat. The variety of the samples was verified with scientists at Main Vegetable Research Station (M.V.R.S.) at Anand Agricultural University, Anand. The initial moisture content for both varieties were 13.93% and 15.04% (w. b.), respectively. Whole and ground (in a laboratory grinder) samples were packed and stored in clean and dry place in the laboratory till experimentation. Distilled water was used for moisture adjustment during conditioning of dried red chilli samples.

Packaging Material

Low density polyethylene (LDPE) having 300 gauge was used as a packaging material during gamma irradiation treatment and storage study. Which was procured from the Hari Agrawal plastic and disposable, Sardargunj, Anand, Gujarat. Size of the packaging bags (thickness 0.05mm) was 7×10 cm and 6× 8 cm was used for whole and powder samples.

Cultures

Aspergillus flavus, *Aspergillus niger*, *Rhizopus*, *Helminthosporium* isolated from samples and maintained in Microbiology laboratory, Department of Food Quality Assurance, Anand AU, Anand.

Drying and Conditioning

Drying and conditioning of samples were done on the basis of initial moisture content. If initial moisture content of dried red chilli higher than the desired moisture level than samples were dried under sun and then conditioned, if required, for the desired amount of moisture content.

$$Q = \frac{A\,(b - a)}{100 - b}$$

Where,
Q = Weight of water to be added (g)
A = Initial weight of sample (g)
a = Initial moisture content of the sample (% w.b.)
b = Final (desired) moisture content of the sample (% w.b.)

After addition of calculated amount of distilled water, the samples were packed in the LDPE bags, mixed thoroughly to ensure uniform distribution of moisture and stored in an incubator at 25 °C for 24 h for moisture equilibrium.

Culture Preparation

Harvesting of Fungi

Potato Dextrose Agar plates were prepared and then the old plates of selected microorganisms were scrapped with the help of nicrome wire loop and fungi was transferred on the fresh prepared the PDA agar plates for the fungi growth. Then prepared plates were kept in an incubator (at 37±2 °C) for growth of selected microorganisms.

Inoculation of *Aspergillus flavus* Spore Suspension in Dried red chilli Samples

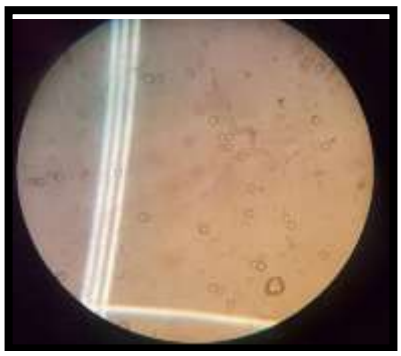
The procedure used by Ferreira-Castro, *et al.* (2007) [7] was followed for the inoculation of spore suspension in the dried red chilli samples.

The spore suspension was prepared by diluting *Aspergillus flavus* in a phosphate buffer solution PBS (1 tablet of phosphate buffer in 100ml distilled water of pH (6.8). After shaking, 100 ml of the PBS was mixed with two drops of Tween 80. The spores were counted using Hemocytometer. The suspension concentration was adjusted to 10^6 spores/ml. Then, 50 g dried red chilli samples were inoculated with 0.5 ml of the fungal spore suspension (using an auto pipette).

Spore Counting using Haemocytometer

The procedure described in Manual of methods for Analysis of foods microbiological testing (FSSAI) 2012 was followed for the spore counting using a Hemocytometer.

Hemocytometer consists of a thick glass microscope slide (1.2x0.8 cm), surrounded by a moat flanked on each side by shoulders 0.1mm higher than plane surface, with rectangular indentations that creates a chamber and a cover glass. Each chamber is engraved with a laser-etched grid of perpendicular lines. The slide and cover glass was carefully cleaned with 70% ethanol to avoid any contamination and counting errors. It was then dried with a sterilized lens paper and the cover glass placed on the shoulders which leaves a depth of 0.1 mm between underside of the cover glass and plane surface. Suspensions were prepared by adding 100 ml of distilled water to 20 g chilli sample in a test tube and thoroughly shacked using a vortex mixture. About 0.1-0.2 ml of suspension from chilli samples or PBS, using an auto pipette (1 ml), was carefully transferred on the slide for uniform spread and covered with cover glass. Proper care was taken that slide neither be overfilled nor under-filled or spillage of liquid into moat and absence of Newton's ring. The hemocytometer then placed under microscope at 10-100X magnification to facilitate localization of the grid. Spores were counted using a hand tally counter on the four large square consisting of 16 small squares at each corner. Following formula was then used for calculation of spore count (numbers per ml or g)



Spore Counting using Haemocytometer

Haemocytometer: spores under microscope

$$\text{Spores per g} = \frac{\text{Counted spores}}{\text{Counted surface area (mm2) x chamber depth (mm) x dilution factor}}$$

Gamma irradiation treatment

Gamma Chamber 5000 (GC-5000), procured from Board of Radiation & Isotope Technology (BRIT), Department of Atomic Energy, Government of India, Mumbai was used for giving treatment to the samples. It is a compact self-shielded Cobalt-60 lab scale gamma irradiator providing an irradiation volume of approximately 5000 cc.

The sample was placed in an irradiation sample chamber located in the vertical drawer inside the lead flask. This drawer can be moved up and down with the help of system of motorized drive which enables precise positioning of the irradiation chamber at the Centre of the radiation field. Radiation field was provided by a set of stationary Cobalt-60 sources placed in a cylindrical cage. The sources were doubly encapsulated in corrosion resistant stainless steel pencils and were tested in accordance with international standards. A mechanism for rotating the sample during irradiation was also incorporated to provide uniform exposure of irradiation on the sample.

Gamma irradiation treatment

The sample (50 g) of each dried red chilli variety (Sannam 4 and Kashmiri) and forms (whole, powdered), having desired moisture content, packed in the LDPE bags was placed in the chamber for the desired dose of irradiation at the dose rate of approximately 9 kGy/h at the center of the chamber. The dose for the samples was controlled by GC-5000 control panel. After applying gamma irradiation to the samples, the samples were stored at room temperature.

Results and Discussion

Microorganisms differ greatly in their resistance to radiation. There are differences in radiation resistances from species to another, and even among strains of the same species. The radiation resistance of microorganisms is measured by the so-called decimal reduction dose (D_{10}-value) which is defined, as the radiation dose (kGy) required killing 90% of the population of a microbe (Roberts, *et al.* 1980) [17]. Therefore, radiation resistance D_{10} values of the potentially pathogenic microorganisms: *Aspergillus flavus*, *Aspergillus niger*, *Rhizopussp* and *Fusarium sp* isolated from chilli samples were determined following method as reported by Abdel-Khalek, (2008) [1].

The fungal microorganisms were enumerated on Potato Dextrose Agar media and spore suspensions (10^6 -10^7 spores/ml) were prepared using physiological saline solutions (0.85% NaCl). D_{10} values of the various fungi were determined using gamma radiation (0.5, 1.0, 2.5, 5.0, 7.5 and 10.0 kGy) dose-response curve and reported in Table 4.2.

Table 1: Average radiation resistance (D_{10} values) of predominant fungi in the samples

Microbial spores	Avg. irradiation D_{10} (kGy)
Aspergillus flavus	1.2
Aspergillus niger	1.1
Rhizopus sp	0.9
Fusarium sp	1.1

From the table, it can be observed that D_{10} values of the various fungi ranged between 1.2 kGy (*A. flavus*) and 0.9 (*Rhizopus sp*). *A. flavus* shown a higher D_{10} value in comparison to *A. niger* and *Fusarium sp* (1.1 kGy). Maity *et al.* (2011) also reported that *A. flavus* requires higher dose to reduce viability to 10% (D_{10}) value in comparison to other major contaminating fungi *Allternaria alternata*, *Trichoderma viride* and *Curvularia* geniculate. Iqbal, *et al.* (2013) reported similarly that the maximum microbial load and aflatoxin in ground and whole chilli was reduced from $6.5x10^7$ to 11 cfu/g and 20±0.91 to 0.5±0.1 µg/kg, respectively, when gamma irradiated at 6 kGy.

Youssef, *et al.* (1999) also observed complete inhibition of fungi by gamma irradiation doses from 4 to 6 kGy in different food and feed products. It can be said that irradiation dose of about 7.2 kGy (considering max D_{10} value 1.2 kGy) required for the acceptable 6 (six) log reductions of the microbial load. McNamara, *et al.* (2003) stated that irradiation may cause direct damage to DNA of the organism through ionization leading to mutations in some cases, in others killing the cell depending on several factors. Further, irradiation also has an indirect effect as a result of radiolysis of cellular water and formation of active oxygen species, free radicals and peroxides causing single and double strand DNA breakages. The current finding also indicated that chilli samples contaminated with *Aspergillus flavus* can be detoxified by gamma-irradiation. *A. flavus* is known for producing poisonous and cancer-causing mycotoxin known as aflatoxins during storage, transportation and food processing (Abdel-Khalek, 2008, Iqbal, *et al.* 2013) [1]. Therefore, only *A. flavus* was used in further study.

Decimal reduction dose D_{10} values (radiation resistance) of the potentially pathogenic microorganisms: *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus sp*and *Fusarium sp* isolated from chilli samples were 1.2, 1.1, 0.9 and 1.1 kGy, respectively. Complete decontamination was observed at the radiation dose of 7.5 kGy in the both varieties and forms of the dried chilli samples having different moisture content (8 to 14%).

From the study, overall it can be concluded that gamma irradiation is promising technology for decontamination of whole and powdered red chilli. The dose of 7.5 kGy was observed to completely eliminate all microorganisms in both forms and varieties studied.

Conclusion

The experiments were designed and conducted with the broad aim of reducing microbial load of dried red chilli using gamma irradiation treatment and quality parameters were evaluated. For the study, bulk samples of dried red chilli, namely Kashmiri and Sannam 4 (locally known as A.D.) were procured from local market of Anand district and stored in LDPE bags (300 gauge). Varieties were verified with scientists at Main Vegetable Research Station (M.V.R.S.) at Anand Agricultural University, Anand. Known population of the spore suspensions (stock solution) was prepared from the preserved microorganism which was isolated from the market samples. Samples of known moisture content and microbial load of both variety and forms were γ irradiated for 2.5, 5.0, 7.5 and 10.0 kGy using gamma radiation research facility at Department of Food Engineering, CFPT&BE, Anand.

From the study, overall it can be concluded that gamma irradiation is promising technology for decontamination of whole and powdered red chilli. The dose of 7.5 kGy was observed to completely eliminate all microorganism in both forms and varieties studied.

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