THE EFFECT OF GAMMA IRRADIATION ON MICROBIOLOGICAL PROPERTIES OF CHILLI POWDER, CHILLI FLAKES (*Capsicum annuum* L.) AND BLACK PEPPER POWDER (*Piper nigrum* L.)

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INTRODUCTION

Spices and herbs are commonly used in food while they also have medicinal properties. Spices and condiments are responsible for the sensory characteristics of food due to the presence of volatiles and fixed oils which are effective even in low quantities (FSSAI, 2015). In addition to the flavour, colour and aroma, spices have the ability to preserve food and beverages. Among the spices, chilli and black pepper have a very important place in the spice industry. Chilli (*Capsicum annuum* L.) belongs to the family of Solanaceae and is locally known as "*Miris"*. The ripen pods of chilli are used to produce chilli flakes and chilli powder. Black pepper (*Piper nigrum* L.) locally known as "*Gam miris"* belongs to the family Piperraceae and the seed is used on an industrial scale.

Fresh red chilli and black pepper are easily perishable. Therefore, many preservation techniques such as drying, vacuum packaging, sealing and adding artificial preservatives have been introduced. However, due to the handling and processing conditions, the spices could be drastically contaminated by various microorganisms. Therefore, gamma irradiation was applied with the main target of eliminating the insect pests and microorganisms in the spices. In order to ensure the consumer safety, wholesomeness, functional and sensorial properties of spices, 10 kGy is the highest absorbed dose that can be applied to a spice while it is necessary to meet legal requirements to apply higher doses (Codex Alimentarius Commission, 2003). The threshold doses for chilli and black pepper are respectively more than 10 and 8 kGy (IAEA, 1992). Among various foods, the most frequently irradiated commodity is the spices (Horváthová, Suhaj, & Polovka, 2007).

In order to measure the effectiveness of the irradiation process, it is necessary to assess the microbiological quality of the irradiated spices. Since chilli powder, chilli flakes and black pepper powder are the most consumed spices at households in Sri Lanka, the current study focused mainly on these spices. In addition, consumers disapprove irradiated food mainly due to the lack of research on the effect of irradiation on the quality aspects of irradiated food commodities like spices in Sri Lanka. Though other European and Asian countries have already standardized their microbial community structure based on gamma-irradiated spices, it has to be standardized according to the Sri Lankan settings by more research findings.

MATERIALS AND METHODOLOGY

Chilli powder, chilli flakes and black pepper powder samples were collected from a mill at Karandeniya a day before the irradiation. The spice samples were weighed and packed separately in a number of sterilized zip lock bags following the sample plan. Control samples of each spice type were separated. Gamma irradiation was done at Sri Lanka Gamma Centre, Sri Lanka Atomic Energy Board, Biyagama using the industrial gamma irradiator. Except the control samples, the rest of the spice samples were irradiated at 1 kGy, 3 kGy, 5 kGy, 7 kGy, 10 kGy doses at the dose rate of 4.17 $Gymin^{-1}$ by the 200 kCi irradiation source. The absorbed dose was measured by using Harwell Amber 3042 V (Amber PMMA) dosimeters.

The total plate count was determined according to the ISO 4833-1:2013 standard method using plate count agar. A quantity of 10 g from the spice sample was mixed with 90 ml of buffered peptone water and decimal dilutions were prepared. 1 ml of each dilution was pipetted into petri dishes before adding the 15 ml plate count agar. After solidification the plates were incubated for 72±3 hours at 30±1 °C. The colonies were counted by using a colony counter.

Yeast and mould counts were determined according to the modified method of ISO 21527-2:2008 using potato dextrose agar. Potato dextrose agar medium plates were prepared and allowed to dry. Then, 0.1 ml of spice homogenate was pipetted into separate petri dishes and spread with sterile spreader. The plates were incubated for 5-7 days at 25 °C and colony counts were taken.

The coliforms were determined according to the ISO 4831:2006 standard method by using the MacConkey broth medium and *E.coli* counts were determined using Eosin methylene blue agar medium (EMB) complying with the ISO 4831:2006 standard method. Each 3 sets of tubes containing MacConkey broth and inverted durham tube, were inoculated with 1 ml of spice homogenate dilutions. These tubes were incubated at 37 ℃ for 24 hours using a water bath. In confirmation step, the tubes with no gas production after 24 hours were re-incubated for 48 hours. The number of positive tubes were recorded at each dilution. One set of positive samples was inoculated in Brilliant green bile broth and placed at 37 ℃ for 24 hours. The total coliform count was taken by using the most probable number (MPN) method.

The samples from the total coliform count test were inoculated in Brilliant green bile broth (BGLB) and placed at 44 ℃ for 24 hours in a water bath to identify fecal coliform. Under the completed step, positive tubes with gas production were counted and fecal coliform count was calculated by using the most probable number (MPN) method.

The fecal coliform positive tubes were subjected to *E.coli* test using Eosin methylene blue agar medium. Samples were inoculated and incubated at 30 ℃ for 24 hours. Microbial analysis was performed with three replicates.

Statistical Analysis

Data analysis of the descriptive and inferential statistics were carried out using Minitab 17 software to determine the mean and standard deviation. One-way ANOVA (Analysis of Variance), Tukey's pair wise comparison test was used to compare the mean values.

RESULTS AND DISCUSSION

Table 1. Effect of different doses of gamma irradiation on the microbial content of selected spices (per gram).

Radiation dose (kGy)	Parameter			
	TPC (CFU/g)	YMC (CFU/g)	TCC (MPN/g)	FCC (MPN/g)
Chilli powder				
0	$2.40\times10^{4}\pm3.31\times10^{3a}$	$2.43\times10^{4}\pm4.04\times10^{3a}$	$9.30\times10^{2}\pm0.00^{a}$	-
1	$1.20\times10^{4}\pm2.99\times10^{3b}$	$1.33\times10^{4}\pm4.16\times10^{3b}$	$1.90\times10^{2}\pm3.46\times10^{1b}$	-
3	$6.77\times10^{3}\pm1.40\times10^{3c}$	$2.33\times10^{2}\pm3.06\times10^{1c}$	$6.13\times10^{1}\pm2.19\times10^{1c}$	-
5	$1.77\times10^{3}\pm8.04\times10^{2d}$	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	-
7	$3.20\times10^{2}\pm3.00\times10^{1d}$	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	-
10	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{d}	-
Chilli flakes				
0	$1.94\times10^{5}\pm4.84\times10^{4a}$	$1.80\times10^{4}\pm2.65\times10^{3a}$	$1.84\times10^{2}\pm7.97\times10^{1a}$	-
1	$8.80\times10^{3}\pm1.00\times10^{2b}$	$1.17\times10^{4}\pm1.53\times10^{3b}$	0.00 ± 0.00^{b}	-
3	$2.13\times10^{3}\pm3.21\times10^{2b}$	$2.90\times10^{2}\pm5.29\times10^{1c}$	0.00 ± 0.00^{b}	-
5	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	-
7	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	-
10	0.00 ± 0.00^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	-
Black pepper powder				
0	$1.19\times10^{6}\pm3.66\times10^{5a}$	$5.67\times10^{5}\pm6.66\times10^{4a}$	$5.97\times10^{2}\pm2.89\times10^{2a}$	$3.07\times10^{1}\pm5.31\times10^{1a}$
1	$5.50\times10^{5}\pm4.58\times10^{4b}$	$1.43\times10^{4}\pm1.15\times10^{3b}$	$7.40\times10^{1}\pm0.00^{b}$	0.00 ± 0.00^{b}
3	$1.07\times10^{5}\pm5.00\times10^{2c}$	$7.00\times10^{3}\pm1.00\times10^{3b}$	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
5	$1.07\times10^{4}\pm3.51\times10^{2c}$	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
7	$1.94\times10^{3}\pm5.00\times10^{0c}$	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
10	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}

TPC: Total Plate Count; YMC: Yeast and Mould Count; TCC: Total Coliform Count; FCC: Fecal Coliform Count

Different letters indicate the significant differences at level P=0.05

According to Table 1, the total plate counts, yeast and mould contents and total coliform counts of chilli powder, chilli flakes and black pepper powder decreased with the increasing irradiation dose. Fecal coliforms were also determined to be $3.07\times10^{1}\pm5.31\times10^{1}$ MPN/g, in black pepper powder samples, whereas no fecal coliforms were detected in chilli powder and chilli flakes. Under the qualitative test for *Escherichia coli* in black pepper powder, black nucleated centres on colonies which denoted the presence of *Escherichia coli* were observed.

According to the statistical analysis (ANOVA), there were significant reductions in the total plate count, yeast and mould count, total coliform count, fecal coliform count and the presence of *Escherichia coli* with 1 kGy gamma irradiation dose in chilli powder, chilli flakes and black pepper powder (P <0.05) (Tukey pairwise comparison test, df=5,17).

The destruction of the microbes present in spices were done according to the basic principle of ionizing radiation. The DNA present in cell nucleus is disrupted by the ionizing radiation by inactivating the active cells. The spontaneous and extensive ionization of gamma irradiation leads to drastic cell disruption (Diehl, 1995).

Zero total plate counts (0.00±0.00 CFU/g) were recorded at 10 kGy for chilli powder and black pepper powder. For chilli flakes a zero total plate count (0.00±0.00 CFU/g) was achieved at 5 kGy. The studies conducted by Abdel-khalek, (1999) on ground paprika and black pepper have shown that the satisfactory reduction of the total plate count could be achieved between the 8-10 kGy dose levels (Abdel-khalek, 1999). The total aerobic count of bacteria was lowered to less than 110 CFU/g by a dosage of 10 kGy (Alshawi, 2016). The bacterial counts in chilli pepper, and black pepper were reduced to below 10^{3}-10^{4} CFU/g by the 5 kGy dose (Munasiri et al., 1987). The doses from 3 kGy to 7 kGy can be used to reduce the viable
cell counts to a satisfactory safety level (Thibbotuwawa, Wijayasiri & Hirimuthugodage, 2017).

Zero yeast and mould counts (0.00±0.00 CFU/g) were achieved by all the spice varieties at 5 kGy irradiation dose. These results were proved by many other research findings. The fungi population in black pepper was reduced up to sterilization level by a dose of less than 6 kGy (Saleh et al., 1988). On a study about the red chilli and black pepper, 5 kGy was sufficient to decrease the mould load by 65%, but the complete elimination was achieved at 10 kGy (Calado et al., 2014). The inactivation doses of yeast and mould of dry spices are reported to be less than 6 kGy (Farkas, 1985).

Chilli powder achieved zero total coliform counts (0.00±0.00 MPN/g) at 5 kGy irradiation dose. At 1 kGy dose, chilli flakes achieved the zero total coliform counts. However, black pepper powder achieved the zero total coliform counts at 3 kGy. These results were supported by several studies. Coliform count of black pepper powder was eliminated at 4 to 10 kGy in a study conducted by Juri et al., (2014) and at 3 kGy in another study by Kwon et al., (1998). Indu et al., (2006) found that 2 kGy was also sufficient to destroy coliforms in red pepper powder.

The fecal coliforms were absent (0.00±0.00 MPN/g) in both the control and all irradiated samples of chilli powder and chilli flakes. However, there were two positive tubes in 10^{-1} dilution of the control sample of black pepper powder according to the completed test of the fecal coliform determination. All fecal coliforms were destroyed with the irradiation treatment of 1 kGy in black pepper powder. According to research, irradiation dose 5 kGy was sufficient to eliminate the *Enterobacteriaceae* family bacteria (IAEA, 1992). In black pepper and red pepper, 5 kGy dose decreased the *E.coli* population to an amount less than 4.40 log
CFU/g and less than 5.20 log CFU/g respectively (Song et al., 2014). According to this study, *E. coli* was not detected in red pepper powder at the dose of 5 kGy irradiation (Juri et al., 2014).

CONCLUSIONS

The application of gamma irradiation on chilli powder, chilli flakes and black pepper powder at 7 kGy, 3 kGy and 7 kGy respectively was sufficient for achieving the microbial safety level for human consumption. Considering chilli powder, chilli flakes and black pepper powder, a dose of gamma irradiation up to 10 kGy could be considered as an effective sterilization method.

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