ENHANCEMENT OF THE EFFICIENCY OF RADIATION STERILIZATION OF GROUND PORK BY THE ADDITION OF NATURAL MATERIALS

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***Abstract*-The objective of this study was to examine the effects of different forms of natural materials on the changes of relative radiation sensitivity (RRS) of *Escherichia coli* and *Listeria monocytogenes* inoculated into ground pork. Garlic, leek, onion, and ginger were prepared as 3 different forms; pressurized (P), freeze dried (FD), and 70% ethanol extracted (EE). The prepared natural materials were subdivided into 2 groups, non-irradiated and irradiated at 5 kGy by gamma ray, before addition into ground pork. The prepared different forms of natural compounds were added 1% concentration (w/w) into radiation-sterilized ground pork and inoculated with *E. coli* and *L. monocytogenes* (10^6 cfu/mL). For *E. coli*, the most efficient material was leek with EE (RRS = 3.887) and followed by ginger and leek with FD (RRS = 3.661 and 3.633, respectively) on non-irradiated. However, when the natural material was irradiation-pasteurized, the ginger with FD showed the highest RRS (4.101). For *L. monocytogenes*, RRS was relatively lower than *E. coli* in general. The most efficient material was onion with P and FD (RRS = 2.133 and 2.077, respectively). Results suggest that addition of natural compound can increase the efficiency of radiation-sterilization process. However, the change of RRS can be different by species of microorganisms and addition forms of natural materials.**

***Index Terms*: natural material; D_{10}; irradiation; relative radiation sensitivity**

I. INTRODUCTION

The combination of natural products and irradiation had a positive effect in increasing the radiosensitization of bacteria (Caillet et al., 2006). Among the natural products, essential oil such as oregano oil from the spices and herbs as antibacterial compounds were effective in controlling the presence of pathogens and/or extending the shelf life of processed foods (Oussalahet al., 2007). This concept can be a useful tool to develop the foods for specific purpose with ultimate safety such as space, military, and immuno-compromized patient foods. Due to the higher irradiation dose (10 kGy or much higher) applied, the sensorial satisfaction of these foods can be reduced by off-odor and off-taste caused from high dose irradiation process. The combination of natural products with irradiation can increase the efficiency of irradiation, thus, a lower dose can be used to meet the same level of safety.

However, to enlarge the application using the method for improvement of efficiency of irradiation process, it is better to use the natural materials within the recipe of manufacturing certain meat products. Korean processed meat products contain different natural additives such as garlic, onion, leek, ginger, green onion, pepper and soy sauce. From the preliminary test, the use of natural form (sliced) of these natural additives did not show significant effect on increasing the radiation sensitivity of processed meat products (Data not shown). Therefore, the objective of this study was to examine the effect of different forms of natural materials on the changes of relative radiation sensitivity of *E. coli* and *L. monocytogenes* inoculated into ground pork.

II. MATERIALS AND METHODS

A. Meat and irradiation

Ground pork was purchased from a local market in Daejeon, Korea in June, 2009 and sealed into polyethylene pouch (2 mL $O_2/m^2/24$ h at 0 °C, Sunkyung Co. Ltd., Korea). For the inoculation test of pathogens, samples were irradiated (35 kGy) to sterilize using a cobalt-60 gamma irradiator (point source AECL, IR-79, MDS Nordion International Co. Ltd., Ottawa, ON, Canada) at the Advanced Radiation Technology Institute, Jeongeup, Korea. The source strength was approximately 320 kCi with a dose rate of 20 kGy/h at 10±0.5 °C.

B. Test pathogens preparation

Escherichia coli (KCTC 41682) and *Listeria monocytogenes* (KCTC 3569) were obtained from Korean Collection for Type Culture (KCTC, Daejeon, Korea). The strains were cultivated at 37°C for 18 h in a tryptic soy broth (Difco, Laboratories, Detroit, MI, USA), and 10 mL cultures of each strain were transferred aseptically to a 50 mL centrifuge tube and vortexed for 10 sec. Each strain was centrifuged (1950 × g for 10 min at 4°C) in a refrigerated centrifuge (VS-5500, Vision Scientific Co., Seoul, Korea). The pellet was washed twice with sterile saline (0.85%), and suspended in saline to a final concentration of approximately 10^9 cfu/mL of the stock inoculum.

***C.* Sample preparation**

Fresh garlic (*Allium sativum* L.), leek (*Allium tuberosum* R.), onion (*Allium cepa* L.), and ginger (*Zingiber officinale*) were purchased from a local market in Daejeon, Korea. Each natural compound was prepared for 3 different forms: (1) pressurized form; each sample (500 g) was washed with tap water and ground using a food mixer (SFM-353NK, SHINIL Co. Ltd., Korea). The sample was pressurized with cloth by hand and the liquid after pressurizing was filtered using Whatmann No. 2 filter paper (Whatmann, Kent, England), then stored at -20°C until used. The liquid form of filtrate was considered as pressurized form. (2) freeze-dried form; each sample (500 g) was washed, ground, and freeze-dried (SFDSF12, Samwon Co. Ltd., Pusan, Korea), and stored at -20°C. (3) 70% ethanol extracted form; each sample (500 g) was extracted three times with 70% ethanol for 12 h in enclosed flask with constant shaking (100 rpm) at room temperature. After filtration with a Whatmann No. 2 filter paper, the residue was re-extracted with additional 200 mL of 70% ethanol for 12 h and then filtered. Ethanol was evaporated using a rotary evaporator (Eyela N-1000, Japan) at 50°C. Then, it was freeze dried. Dried extracts were placed in sealed bottles and stored at -20 °C before use. The dried powders were dissolved in water (10% w/w) prior to use. To see the effect of contamination of additive, the half of the prepared natural compounds with different forms were irradiated at 5 kGy as the same method previously described (section 2.1) to reduce the original number of microorganisms present. The other half was used without irradiation.

***D.* Inoculation of pathogens, irradiation, and D_{10} value**

The prepared 3 different forms of natural compounds, both irradiated at 5 kGy or none, were mixed with radiation-sterilized ground pork (50 g) at a concentration of 1% (w/w, final concentration). The control sample without any additive was also prepared. The sample was sub-divided into portions each have 5 g in sterile polyethylene pouch bag (8 x 10 mm), and inoculated with *L. monocytogenes* and *E. coli*. The test culture suspension (50 μL) was uniformly and aseptically inoculated in different areas on the samples and mixed to achieve uniform dispersal at the desired concentration throughout the sample for 5 min in enclosed polyethylene bag. The bags were sealed and the inoculated samples were stored at 4 °C for approximately 12 h prior to irradiation. Irradiation of sample was done as the same method as previously described (section 2.1). The absorbed doses applied were 0, 0.5, 1, 2, 3, and 5 kGy to obtain D_{10} values, the exposure time required to inactivate 90% of a population from survival curves. After calculation of D_{10} values, relative radiation sensitivity (RRS) was calculated as D_{10} of control / D_{10} of sample with additives (Lacroix et al., 2009).

***E.* Microbiological analysis**

After irradiation treatment, samples were blended with sterile saline using a stomacher (BagMixer ® 400, Interscience Ind., St. Nom, France) for 2 min. Then, a series of decimal dilution was prepared with sterile saline and each diluent (0.1 mL) was spread in tryptic soy agar (Difco Laboratories). The plates were incubated at 37 °C for 48 h and microbial counts were expressed as log CFU/g.

***F.* Statistical analysis**

Experiment was conducted as 3 independent trials with 2 observations for treatment combinations per each trial. Statistical analysis was performed by one-way Analysis of Variance (ANOVA), and when significant differences were detected, the differences among the mean values were identified by Student-Newman-Keul's multiple range test using SAS software with the confidence level at $P < 0.05$ (SAS, Release 8.01, SAS Institute Inc., Cary, NC). Mean values and standard error of the means are reported.

III. RESULTS AND DISCUSSION

All natural materials were effective in improving the radiation inactivation efficiency of *E. coli*, indicating 2-4 times lower D_{10} values when compared with control (Table 1). Within the same natural materials, the ED was the best to increase RRS except for ginger, showing the highest activity in a FD. The most effective RRS was found in the ED of leek followed by FD forms of leek and ginger. However, when the additives were irradiated for 5 kGy before the addition into ground pork, the effectiveness was not differ between the forms of garlic and ginger. FD of ginger showed the highest RRS (4.101) in this test.

The D_{10} value of *L. monocytogenes* in ground pork was 0.797 (Table 2). Similarly, addition of natural compounds (1%) increased the radiation sensitivity of *L. monocytogenes* approximately 1–2 times. In general, the P or FD of natural materials had higher RRS with minor exceptions. However, RRSs were not as high as when *E. coli* was tested. Furthermore, the addition of ED of onion and ginger showed higher D_{10} values than that of control when the additives were mixed without irradiation pasteurization.

The natural materials tested in the present study may possess antimicrobial and/or antifungal properties majorly due to the presence of phenolic compounds (Dorman and Deans, 2000). The mechanism of antimicrobial activities by phenolic compounds may involve multiple modes of action. Shan et al. (2007) suggested that phenolic compounds can degrade the cell wall, disrupt the cytoplasmic membrane, cause leakage of cellular components, change fatty acid and phospholipid constituents, influence the synthesis of DNA and RNA and destroy protein translocation. The phenolic compounds also involve a sensitization of the phospholipid bilayer of the cell membrane, causing an increase in permeability and leakage of vital intercellular constituents (Kim et al., 1995) or impairment of bacterial enzyme (Wendakoon and Sakaguchi, 1995). Lacroix et al. (2009) reported that in the presence of Spanish oregano essential oil, the radiation D_{10} values of *E. coli* O157:H7 and *L. monocytogenes* decreased from 0.27 and 0.36 to 0.087 and 0.13 kGy, respectively (RRS = 3.1 and 2.75, respectively). The decrease of intracellular ATP concentration of *E. coli* and *L. monocytogenes* by the addition of the essential oil can be associated with the ability of the essential oil to disrupt the membranes of the bacterial cell wall and cause lysis (Mahrour et al., 2003).

IV. CONCLUSION

In conclusion, the present study demonstrates that the combination of natural materials for food manufacturing and irradiation was useful in improving the efficiency of radiation sterilization. However, the different forms of natural materials and kinds of pathogen species may produce different effects. This combination technology can be applied for manufacturing high-dose irradiated foods with ultimate safety such as space or immune-compromised patients' foods because the increased radiation efficiency may decrease the required dose, resulting in the reduction of sensory deterioration caused by high-dose irradiation.

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Table 1. Relative radiation sensitivity of *Escherichia coli* (KCTC 41682) in ground pork with different forms of natural materials at 1% concentration (w/w)

Irradiation dose (kGy) for additive	Natural compound		D_{10} (kGy)[1)]	Relative radiation sensitivity[2)]
	Control		0.898	1.00
0	Garlic	Pressurized	0.517	1.736z[3)]F[4)]
		Freeze dried	0.466	1.926yF
		70% EtOH extract	0.270	3.323xCD
	Leek	Pressurized	0.262	3.429yBC
		Freeze dried	0.247	3.633yB
		70% EtOH extract	0.321	3.887xA
	Onion	Pressurized	0.473	1.899yF
		Freeze dried	0.534	1.680yF
		70% EtOH extract	0.287	3.124xD
	Ginger	Pressurized	0.354	2.540yE
		Freeze dried	0.245	3.661xB
		70% EtOH extract	0.376	2.390yE
5	Garlic	Pressurized	0.328	2.737AB
		Freeze dried	0.389	2.311B
		70% EtOH extract	0.248	3.623AB
	Leek	Pressurized	0.251	3.579xAB
		Freeze dried	0.297	3.025yAB
		70% EtOH extract	0.341	2.634zAB
	Onion	Pressurized	0.412	1.636yC
		Freeze dried	0.395	2.276xAB
		70% EtOH extract	0.262	3.434xAB
	Ginger	Pressurized	0.235	3.825AB
		Freeze dried	0.219	4.101A
		70% EtOH extract	0.254	3.541AB

1)Radiation dose required reducing 90% population calculated from the results of 0, 0.5, 1, 2, and 5 kGy of irradiated sample.

2) D_{10} of control/D_{10} of the sample mixed with natural materials.

3)Values with the different letters (x-z) within the same natural material with different forms in the same irradiation dose differ significantly ($P < 0.05$).

4)Values with the different letters (A-F) within the same prepared forms in the same irradiation dose differ significantly ($P < 0.05$).

Table 2. Relative radiation sensitivity of *Listeria monocytogenes* (KCTC 3569) in ground pork with different forms of natural materials at 1% concentration (w/w)

Irradiation dose (kGy) for additive	Natural compound		D_{10} (kGy)[1)]	Relative radiation sensitivity[2)]
	Control		0.797	1.000
0	Garlic	Pressurized	0.472	1.697xB
		Freeze dried	0.672	1.186zCD
		70% EtOH extract	0.550	1.449yBC
	Leek	Pressurized	0.552	1.445xCB
		Freeze dried	0.538	1.482xBC
		70% EtOH extract	0.776	1.027yD
	Onion	Pressurized	0.374	2.133xA
		Freeze dried	0.659	1.209yCD
		70% EtOH extract	0.800	0.996yD
	Ginger	Pressurized	0.662	1.203xCD
		Freeze dried	0.635	1.255xCD
		70% EtOH extract	1.205	0.662yE
5	Garlic	Pressurized	0.479	1.663yBC
		Freeze dried	0.593	1.343zCD
		70% EtOH extract	0.425	1.871xAB
	Leek	Pressurized	0.772	1.033zD
		Freeze dried	0.604	1.321xCD
		70% EtOH extract	0.654	1.218yCD
	Onion	Pressurized	0.379	2.103A
		Freeze dried	0.384	2.077A
		70% EtOH extract	0.511	1.560BC
	Ginger	Pressurized	0.500	1.593BC
		Freeze dried	0.567	1.406BCD
		70% EtOH extract	0.533	1.496BCD

[1)]Radiation dose required reducing 90% population calculated from the results of 0, 0.5, 1, 2, and 5 kGy of irradiated sample.

[2)] D_{10} of control/D_{10} of the sample mixed with natural materials.

[3)]Values with the different letters (x-z) within the same natural material with different forms in the same irradiation dose differ significantly ($P < 0.05$).

[4)]Values with the different letters (A-F) within the same prepared forms in the same irradiation dose differ significantly ($P < 0.05$).

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