Sterilization of Freeze Dried Manila Clam (*Ruditapes philippinarum*) Porridge for Immuno-Compromised Patients

Beom-Seok Song[1] and Jae-Nam Park[2,*]

[1]*Team for Radiation Food Science & Biotechnology, Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, 29, Geumgu-gil, Jeongeup-si, Jeollabuk-do 56212, Republic of Korea*
[2]*Department of Food Science & Nutrition, Songwon College, 73, Songam-ro, Nam-gu, Gwangju 61756, Republic of Korea*

Abstract - This study was conducted to evaluate the combined effect of gamma irradiation and different conditions (vacuum packaging, antioxidant and freezing) on the microbiological and sensory characteristics of freeze dried Manila clam porridge (MCP) for immuno-compromised patient food. MCP can be sterilized at 1 kGy to 10 kGy. The initial counts of total aerobic bacteria and yeast molds in the non-irradiated MCP were 2.4±0.5 and 1.2±0.3 log CFU g^{-1}, respectively, but gamma irradiation significantly decreased the total aerobic bacteria to below the detection limit (1 log CFU g^{-1}) (5 kGy). Moreover, gamma irradiation effectively eliminated yeasts/molds at dose below than 1 kGy. However, gamma irradiation accelerated the increase of lipid oxidation and therefore, decreased the sensory characteristics of MCP as irradiation dose increased. To improve the sensory qualities of gamma irradiated MCP, combination treatment (vacuum packaging, 0.1% vitamin C) were applied. There was no significant difference in the overall acceptance scores between the combined-treatment sample (5.6 points) and the non-irradiated samples (6.0). The results indicate that combination treatment (vacuum packaging, 0.1% vitamin C) may help to maintain the quality of MCP. Therefore, it considered that irradiation of MCP with combined treatment and this is an effective method for the consumption as a special purpose food such as for space travel or immuno-compromised patients.

Key words : Special food, Manila clam, Patient food, Gamma irradiation, Sterilization

INTRODUCTION

Traditionally, more than 200 types of porridges are known to exist in Korea. These porridges have been recognized as a healthy food by including diverse food ingredients such as manila clam, abalone, sweet pumpkin, mushroom, meats, etc. Manila clam porridge is particularly popular for the diet of the elderly, and the infirmed, and as a breakfast food because it is nutritious, and easily digestible (Lee and Jurn 2000). The conventional processing to prepare Manila clam porridge is to cook Manila clam in water with white rice and a variety of vegetables such as carrots, onions, ginsengs, and mung bean by thermal treatment. However, ordinary thermal treatment used for cooking cannot eliminated all microorganisms present in Manila clam porridge and one of the reasons is due to the presence of thermo-stable bacteria contaminated in ingredients such as rice and other vegetables (Kim *et al*. 1996; Niemira *et al*. 2002).

On the other hand, food irradiation has been suggested as an effective sanitary tool for sterilizing foods because this technology inactivates the pathogenic and spoilage microorganisms, and also reduces enzymatic activity in the final products without compromising foods quality (IAEA 2009). Since food irradiation was applied for the safety control of

* Corresponding author: Jae-Nam Park, Tel. +82-62-360-5973, Fax. +82-62-360-5971, E-mail. rotc4151@songwon.ac.kr

harmful microorganisms contaminated in foods, the technology has been used extensively on an industrial scale in many countries (Thayer 1986; Kyzlink 1990). However, as several adverse effects (lipid oxidation, softening, etc.) is accompanied by food irradiation (Lee *et al*. 2005), combined treatment of food additives or freezing with the technology has been attempted to reduce the deterioration of the sensory quality.

Therefore, in this study, we applied gamma-irradiation and combined treatment of vacuum packaging and vitamin C for the preparation of Manila clam porridge as a special purpose food (immune-compromised patients) with acceptable sensory quality. For this goal, effect of gamma-irradiation and combined treatment (vacuum packaging and vitamin C) on the change of the microbiological and sensory characteristics of Manila clam porridge was investigated.

MATERIALS AND METHODS

Sample preparation

Clam broth (actually broth from clams) was prepared by boiling water for 30 min with salt (2%). For Manila clam porridge (MCP), several cooked clams (20 g), and various ingredients (ginseng, mung beans, mushroom, carrot, and welsh onion, about 15 g in total) with pre-soaked (1 h) glutinous rice (50 g) were put into a stainless steel pot. The pot was heated on a hot plate at 200°C for 10 min, with frequent stirring. The cooked MCP was transferred to a glass container and left to cool at room temperature.

After cooling, samples were placed in a 90-mm diameter Petri dish, flattened and frozen in a deep freezer (Model 917; Forma Scientific Inc. OH) at −70°C for 24 h. The frozen samples were freeze-dried using a freeze-drying system (Model FD-5505P, Ilshinlab Co., Seoul, Korea). Finally, the sample was gamma-irradiated.

Irradiation treatment

Samples were irradiated by gamma rays of 1, 3, 5, 7, and 10 kGy with a dose rate of 10 kGy h^{-1} at room temperature (about 15 to 20°C), respectively, at the Korea Atomic Energy Research Institute (Jeongeup, ROK). The source strength was approximately 320 kBq and the actual dose was within 2% of the target dose. The absorbed dose was measured using the alanine-EPR dosimetry system (ISO/ASTM 51607 : 2003). After irradiation, the samples were stored at room temperature.

Microbial analysis

For quantification of microbial growth, samples diluted with 0.1% saline water (1 : 10) were placed in sterilized stomacher bags (20 × 25 cm; Sunkyung Co., Seoul, South Korea) and homogenized for 1 min using a stomacher (Model 400, Tekmar Co., Los Angeles, CA, USA). The diluted solution (1 ml) was then placed on a plate count agar (PCA, Difco Co., Detroit, MI, USA) for determination of the total bacteria content, followed by addition to a 1 ml dilution solution in a 9 ml thioglycollate medium (Difco Co.) to confirm the sterilization. The yeast and mold contents were determined using potato dextrose agar (PDA, Difco Co.) with a diluted solution (1 ml). PCA plates and thioglycollate medium tubes inoculated with samples were incubated at 35°C aerobically for 48 h and 7 days, respectively, and PDA plates inoculated with samples was incubated at 25°C for up to two days. The colonies in all petri dishes were counted, revealing 30∼300 colonies in each dish.

Measurement of TBARS

In order to measure lipid oxidation of MCP, 2-Thiobarbituric acid (TBARS) measured as described by Ahn *et al*. (1999) to determine the lipid oxidation of the MCP. The sample (5 g) was homogenized in a 50 ml centrifuge tube with 50 μl of butylated hydroxyanisol (BHTl) and 15 ml of distilled water, using a homogenizer (D-91126, Heidolph Instruments, Schwabach, Germany). One-milliliter aliquots of the homogenates were mixed with 3 ml of 2-thiobarbiutric acid (20 mM TBA in 15% trichloroacetic acid), heated in boiling water (100°C) for 20 min, and cooled in ice water for 5 min. The cooled mixture was centrifuged for 10 min at 2,500 × g, using a UNION 5 KR centrifuge (Hanil Science Industrial, Co., Ltd., Incheon, Korea). The absorbance of the supernatant was measured at 532 nm using a UV 1600 PC spectrophotometer (Shimadzu, Nagoya, Japan), and was reported as mg of malondialdehyde per kg.

Sensory evaluation

The sensory properties of freeze dried MCP were evaluated using a descriptive 7-point scale (where 1 = extremely dislike or weak, 2 = dislike moderately, 3 = dislike slightly,

4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, and 7 = extremely like or strong) with 10 taste panelists. Sensory evaluation of the samples was conducted by 10 panels (25~40 ages, 5 males and 5 females). A trained ten-member panel consisting of researchers from the department of Advanced Radiation Technology Institute at Korea Atomic Energy Research Institute in Korea evaluated the irradiated freeze dried MCP. An orientation session was conducted before their participation. To research the consumer perception, the appearance, flavor, taste, texture, and overall acceptance were evaluated. In addition, saltiness, fishy, flavor, and off-flavor were tested to confirm the intensity of sensorial characteristics. Water was provided to the panelists for rinsing the mouth between sample testing.

Statistical analysis

The samples were analyzed in triplicate, and all results were expressed as the mean ± standard deviation (SD). The data for TBARS and sensory evaluation were analyzed using general linear models in SAS system version 9.2 software (SAS Institute Inc., Cary, NC, USA). Differences between means were considered significant when $p \leq 0.05$.

RESULTS

Microbiological evaluation

The activation of the microbial population on freeze dried MCP with different doses of gamma irradiation is shown in Table 1. The total aerobic bacteria of freeze dried MCP was 2.4 log CFU g^{-1} before irradiation, but populations in the sample decreased to below the detection limit (1 log CFU g^{-1}) after irradiation at 5 kGy, and 1 kGy gamma irradiation eliminated yeasts/molds. In addition, MCP was sterilized when it was irradiated at 5, 7, and 10 kGy. Gamma irradiation with 5~10 kGy decreased the number of spore forming bacilli in dried foods to below the detection limit (2 log CFU g^{-1}) in a study conducted in Egypt (El-Zawahry *et al*. 1991).

TBA values and sensory characteristics

Both microbial safety and taste are very important factor for developing foods for immuno-compromised patients. The TBA values and sensory characteristics of the MCP samples are shown in Table 2. TBA values of all samples were increased by gamma irradiation. In general, it is known that irradiation induces lipid oxidation and oxidation progresses during storage (Kanatt *et al*. 1998; Byun *et al*. 1999). As supported by our data, lipid oxidation is generally increased during the irradiation process, and different physicochemical treatments can also retard irradiation-dependent lipid oxidation. The control sample (non irradiated) showed

Table 1. Effects of gamma irradiation on the total aerobic bacteria, yeast & molds, sterilization and TBA value of dried Manila clam porridge

Irradiation dose (kGy)	Total aerobic bacteria (log CFU g^{-1})	Yeast & molds (log CFU g^{-1})	Sterilization
0	2.4 ± 0.5	1.2 ± 0.3	+
1	1.5 ± 0.2	ND	+
3	1.1 ± 0.1	ND	+
5	ND	ND	−
7	ND	ND	−
10	ND	ND	−

Values are mean ± standard deviation (n = 6).
ND: Not Detected within the detection limit < 1 log CFU g^{-1}. + (positive): Bacterial growth was detected. − (negative): Bacterial growth was not detected.
[1)]MA: Malondialdehyde, [2)]a~e values with different letters a column mean significant differences ($p < 0.05$).

Table 2. Sensory properties of dried Manila clam porridge immediately after gamma irradiation

Dose (kGy)	TBA value (μg MA[1)] g^{-1})	Attributes					
		Color	Flavor	Taste	Texture	Overall acceptability	Off flavor
0	21.9 ± 0.6[e2)]	5.5 ± 0.6[NS]	5.8 ± 0.5[a2)]	6.0 ± 0.8[a]	5.8 ± 0.5[a]	5.8 ± 0.5[a]	1.0 ± 0.1[e]
1	24.8 ± 0.2[d]	5.8 ± 0.5	5.0 ± 0.1[ab]	4.5 ± 1.2[b]	5.5 ± 1.0[a]	5.0 ± 0.8[ab]	2.0 ± 0.3[d]
3	25.1 ± 0.7[cd]	5.5 ± 0.6	5.3 ± 0.5[ab]	4.0 ± 0.8[bc]	5.3 ± 0.9[ab]	4.8 ± 0.5[bc]	3.3 ± 0.5[c]
5	25.9 ± 0.5[c]	5.5 ± 0.6	4.3 ± 0.5[bc]	3.5 ± 0.6[bc]	4.8 ± 0.5[ab]	4.0 ± 0.1[cd]	3.5 ± 0.6[bc]
7	27.3 ± 0.9[b]	5.3 ± 1.0	4.3 ± 1.3[bc]	3.0 ± 0.8[c]	4.3 ± 0.5[bc]	3.5 ± 0.6[de]	4.3 ± 0.5[b]
10	30.4 ± 0.5[a]	5.3 ± 0.9	3.5 ± 1.0[c]	2.8 ± 0.9[c]	3.5 ± 0.6[c]	2.8 ± 1.0[e]	5.5 ± 1.0[a]

Values are mean ± standard deviation (n = 10), NS: Non-significantly.
[1)]MA: Malondialdehyde. [2)]a~e values with different letters a column mean significant differences ($p < 0.05$).
Score scale: 1 (very bad) to 7 (very good).

Table 3. Evaluation of sensory properties of dried Manila clam porridge combined with packaging, vitamin C (0.1%), and irradiated different temperature conditions

Sample			Attributes				
			Color	Taste	Texture	Overall acceptability	Off flavor
Non-irradiated			5.5±0.6^{NS}	6.0±0.8$^{a1)}$	6.0±0.5^{a}	6.0±0.5^{a}	1.0±0.1^{d}
Irradiated at 5 kGy	Aerobic	Room temperature	5.0±0.5	3.0±0.6^{d}	4.0±0.5^{c}	3.2±0.5^{d}	2.8±0.4^{a}
		In ice	5.3±0.5	4.9±0.3^{bc}	4.9±0.9^{b}	4.0±0.3^{c}	2.1±0.3^{b}
		In dry ice	5.1±0.4	5.1±0.5^{b}	4.9±0.6^{b}	5.1±0.4^{b}	1.9±0.4^{bc}
	Vacuum	Room temperature	5.2±0.1	4.5±0.5^{c}	4.7±0.5^{bc}	4.5±0.7^{bc}	2.2±0.4^{b}
		In ice	5.4±0.7	5.3±0.8^{ab}	4.8±0.7^{b}	5.2±0.2^{b}	1.5±0.3^{c}
		In dry ice	5.4±0.5	5.5±0.4^{ab}	5.2±0.3^{ab}	5.6±0.5^{ab}	1.1±0.2^{d}

Values are mean ± standard deviation (n = 10), NS: Non-significantly.
[1)]a~d values with different letters a column mean significant differences ($p < 0.05$).
Score scale: 1 (very bad) to 7 (very good).

Table 4. Microbial population of dried Manila clam porridge after gamma irradiation of 5 kGy during storage period

Microorganisms (log CFU g^{-1})	4°C		25°C		35°C	
	0 day	90 day	0 day	90 day	0 day	90 day
TAC[1)]	ND[2)]	ND	ND	ND	ND	ND
Coliform[3)]	ND	ND	ND	ND	ND	ND
Staphylococci[4)]	ND	ND	ND	ND	ND	ND
Salmonella[5)]	ND	ND	ND	ND	ND	ND
Yeast and molds[6)]	ND	ND	ND	ND	ND	ND
Bacillus cereus[7)]	ND	ND	ND	ND	ND	ND

[1)]Total aerobic count, plate count agar (Difco) incubated at 35°C for 48 h, [2)]ND: not detected within detection limit < 1 log CFU g^{-1}, [3)]Desoxycholate lactose agar (Difco) incubated at 35°C for 20 h, [4)]Coagulase positive *Staphylococci* and coagulase mannitol agar (Difco) incubated at 35°C for 24 h. [5)]Triple sugar iron agar (Difco) incubated at 35°C for 24 h, [6)]Potato dextrose agar (Difco) incubated at 25°C for 72 h, [7)]Mannitol egg yolk polymyxin agar (Difco) incubated at 30°C for 48 h.

the highest sensory score among all the samples. The flavor, taste, texture, and overall acceptance scores of the samples significantly decreased following irradiation in comparison with the control samples. Moreover, while MCP was considered adequately sterilized when it was irradiated at 5 kGy (Table 1), irradiation doses of over 5 kGy induced an off-flavor and deteriorated sensory qualities of MCP.

Sensory evaluation of gamma-irradiated MCP in combined treatment

The sensory properties of MCP irradiated under different temperature conditions are shown in Table 3. When concentration of vitamin C was increased up to 0.1%, the overall sensory characteristics were increased (data not shown in a tabular form). It has been reported that vitamin C can function as a radical scavenger that prevents the negative effect on sensory characteristics. In the results of our sensory evaluation, non-irradiated MCP scored 5.5 to 6.0 on a 7.0 point scale. Combinations of irradiation and vacuum packaging have a potential for improving the quality of MCP. Moreover, with a decrease in irradiation temperature, all of the preferred attributes increased with irradiated MCP. In particular, the overall acceptance of the samples irradiated in dry ice had similar scores as non-irradiated samples at 5.6 (in dry ice) and 6.0 (control). In addition, the taste, texture, and off flavor were reduced among the lower temperature samples.

Microbial safety of gamma-irradiated MCP in combined treatment

The evaluation of the microorganisms in MCP sample during storage is shown in Table 4. The safety of patient food is an important matter to the immune-compromised patients as food poisoning due to harmful microorganisms can be fatal. The result shows that the gamma-irradiated MCP was completely sterilized by 5 kGy gamma irradiation in every condition, which means the MCP satisfied the sterilized for immune-compromised patients food. Also, the future research will include anaerobic bacteria assessment.

DISCUSSION

Microorganisms were not detected (< 1 log CFU g^{-1}) in MCP after 5 kGy of gamma irradiation. The results indicate that commercial freeze dried porridge products were not safe enough for immuno-suppressed consumer such as those who have HIV or AIDS, or who are undergoing chemotherapy or radiation therapy for cancer (Todd *et al*. 1999). Gamma irradiation technology provides the possibility to use MCP products for special purpose such immuno-compromised patients of people who need a product with ultimate safety. The deterioration of flavor and taste is related mainly to lipid oxidation, and can also be attributed to amino acids and/or carbohydrate breakdown (Diehl 1981; Lacroix *et al*. 2004). We also found that the intensities of off-flavor were increased with a rising dose by gamma irradiation. The overall acceptance of sterilized MCP (5 kGy gamma irradiation) scored 4.0 (acceptable) at the 7 point scale. However, gamma irradiation-sterilized MCP had lower sensory qualities then the non-irradiated sample. Therefore, it is necessary to improve the sensory qualities of gamma irradiation-sterilized MCP for consumption immuno-compromised patients. Irradiating in a frozen state has been successfully used to overcome the problems of MCP requiring sterilized doses of radiation. The temperature during irradiation is important because the initial ionization, excitation event, and the reactions of the active species are dependent on the temperature (Swallow 1997). The mobility of the free radicals is reduced as the temperature falls, and thus their ability to interact is reduced (Shultz *et al*. 1977; Raffi and Agnel 1983). Thus, undesirable sensory changes are minimized. From these results, it is considered that the 5 kGy irradiation of MCP vacuum packaging under frozen conditions maintains the acceptable sensory properties for the consumption by immuno-compromised patients.

CONCLUSION

In this study, a combination treatment with radiation technology was used to prepare freeze dried MCP, as a food for immuno-compromised patients. The MCP sample treated with 0.1% of vitamin C, vacuum packaging, and gamma-irradiated at 5 kGy under a frozen state (dry ice) showed higher sensory scores than just irradiation. The results suggest that combined treatment with irradiation can enable the incorporation of MCP in the diets of immuno-compromised patients.

ACKNOWLEDGMENT

This work was supported by the Nuclear Research & Development Program of the Korea Science and Engineering Foundation, through a grant from by the government of the Republic of Korea (2012M2A2A6011320).

REFERENCES

Butterweck JS. 1995. Sterile diets for the immuno-compromised: is there a need? *Radiat. Phys. Chem.* **46**:601-604.

Byun MW, Lee JW, Yook HS, Lee KH and Kim KP. 1999. The improvement of color and shelf life of ham by gamma irradiation. *J. Food Prot.* **62**:355-369.

Diehl F. 1981. Effects of combination processes on the nutritive value of food. Combination processes in the food irradiation. 349-366 pp. International Atomic Agency, Vienna, Austria.

El-Zawahry YA, Aziz NH and El-Fiyly MZ. 1991. Incidence of toxic and pathogenic microorganisms in different Egyptian and Saudi Arabian food commodities and their decontamination by gamma irradiation. *Egypt J. Microbiol.* **26**:267-282.

Hanekom SM, Vermeulen EE and Oldewage-Theron W. 2010. Food safety risk factors in a hospital food service unit serving low microbial diets to immuno-compromised patients. *Afr. J. Food Agric. Nutr. Dev.* **10**(9):4000-4015.

IAEA. 2009. Irradiation to Ensure the Safety and Quality of Prepared Meals. International Atomic Energy Agency, Vienna.

Kanatt SR, Chawla SP, Chander R and Sharma A. 2006. Development of shelf-stable, ready-to-eat (RTE) shrimps (*Penaues indicus*) using γ-radiation as one of the hurdles. *LWT - Food Sci. and Technol.* **39**:621-626.

Kim KO, Kim HY and Lee YC. 1996. Optimization of freeze dried instant rice production for infant foods. *Food Sci. Biotechol.* **5**:14-20.

Kyzlink V. 1990. Principles of food freservation. *Dev. Food Sci.* **22**:337-355.

Lacroix M, Ouattara B, Saucier L, Giroux M and Smoragiewicz W. 2004. Effect of gamma irradiation in presence of ascorbic acid on microbial composition and TBARS concentration of ground beef coated with an edible active coating. *Radiat. Phys. Chem.* **71**:71-75.

Lee HJ and Jurn JI. 2000. Research of kinds of rice porridges

and recipes of it. *Korean J. Food and Nurt.* **13**:281-290.

Lee JW, Park KS, Kim JG, Oh SH, Lee YS, Kim JH and Byun MW. 2005. Combined effects of gamma irradiation and rosemary extract on the shelf-life of a ready-to-eat hamburger steak. *Radiat. Phys. Chem.* **72**:49-56.

Niemira BA, Fan X and Sommers CH. 2002. Irradiation temperature influences product quality factors of frozen vegetables and radiation sensitivity of inoculated *Listeria monocytogenes*. *J. Food Protect.* **65**:1406-1410.

Raffi J and Agnel J. 1983. Influence of the physical structure of irradiated starches on their electron spin resonance spectra kinetics. *J. Phys. Chem.* **87**:2369-2373.

Shultz GW, Cohen JS, Mason VC and Wierbicki E. 1997. Flavor and textural changes in radappertized chicken as affected by irradiation temperature, NaCl and phosphate additions. *J. Food Sci.* **42**:885-889.

Swallow AJ. 1997. Chemical effects of irradiation. *In*: Elias PS, Cohen AJ (eds.), Radiation chemistry of major food components, Elsevier, Amsterdam, 5-20 pp.

Thayer WT, Lachica RW, Huhtanene CN and Wierbick E. 1986. Use of irradiation to ensure the microbiological safety of processed meats. *Food Technol.* **40**:159-171.

Todd J, Schmidt M, Christain J and Williams R. 1999. A low-bacteria diet for immunocompromised patients. *Cancer Pract.* **7**:205-207.

Received: 21 September 2016
Revised: 27 October 2016
Revision accepted: 1 November 2016