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Combination Effect of Modified Atmosphere Packaging and Electron Beam Irradiation on the Oxidative and Microbiological Stability of Ground Pork during Storage

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공기 조절 포장과 전자선 조사의 병용이 분쇄돈육의 저장 중 산화와 미생물적 안정성에 미치는 영향

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Abstract

Ground pork was packaged(purged) with modified atmosphere (N_2 and CO_2) and irradiated with the electron beam in order to find out whether modified atmosphere packaging (MAP) inhibit the microbial growth and lipid oxidation development caused by electron beam irradiation. After packaging and irradiation, ground pork was stored at 4℃ for 6 days and −15℃ for 3 months, and periodically the microbial counts and the thiobarbituric acid reactive substances (TBARS) for the determination of lipid oxidation were measured. The inhibition of growth of total aerobic bacteria and mesophiles was confirmed when the ground pork was irradiated with the electron beam dose of 1.5 and 3.0 kGy. The N_2 or CO_2 purging alone was also effective in reducing the development of lipid oxidation of ground pork during storage at 4 and −15℃. The combination of electron beam irradiation(1.5 and 3.0 kGy) with MAP (N_2 or CO_2) was effective to inhibit the growth of total aerobic bacteria and mesophiles, and retard the lipid oxidation of ground pork during storage at 4℃ for 6 days and −15℃ for 3 months.

Key words : ground pork, modified atmosphere packaging, electron beam irradiation, microbial growth, lipid oxidation development.

Introduction

The pasteurization effect of electron beam irradiation, along with γ-ray irradiation, in foods is well reported (Bagorogoza et al., 2001; Farkas, 1998; Johnson and Marcott, 1999; Kim et al., 1998; Kwon et al., 2001; Lee et al., 1998; Olson, 1998; Shamsuzzanman et al., 1995; Thayer and Rajkowski, 1999; 이, 1995). Their other effects includes destruction of insects, inactivation of parasites, delaying of ripening and prevention of sprouting (Olson, 1998). With these reasons, the use of irradiation of foods is increasing throughout the world. However, unlike the irradiation of γ-ray, electron beam irradiation does not induce radioactivity, requires a few seconds for irradiation. Its additional advantage is no temperature change occurring after the treatment and this method is a very environment-friendly mean to accomplish the purposes mentioned above. The utilization of electron beam irradiation in foods is expected to increase in the future for these reasons. In December 1999, Food Safety & Inspection Service (FSIS) of USDA allowed the use of ionizing radiation in the refrigerated and frozen meat to increase the shelf life of the product. In addition, in May

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2000, a company in Minnesota marketed electron beam irradiated frozen beef patty and the consumers' response was very positive (Hermelstein, 2000a). In 2001, Surebeam Corp. which is a major manufacturer of electron beam accelerator in the U. S. decided to provide a 10 year, $ 10-million worth of 2 linear electron beam accelerators and 1 X-ray instruments to Texas A&M university for food use only (Hermelstein, 2000a, 2000b). This alliance is going to stimulate the research activities in this field even further in the near future.

Although the inhibition of growth of bacteria is well established, it is reported that electron beam irradiation promoted the lipid oxidation in foods during storage (Ahn et al., 1998a, 1998b; Ahn et al., 1999; Du et al., 2001; Hansen et al., 1987; Heath et al., 1990; Hwang and Maerker, 1993; Nam et al, 2001; 이, 1995). It is known that the irradiation produces peroxides or various radicals (Du et al., 2001; Nam et al, 2001) which propagates lipid oxidation. Therefore, in order to fully utilize the advantages of electron beam irradiation, the control of lipid oxidation is the first problem that must be solved. This research was carried out to find out whether the combination of modified atmosphere packaging (MAP, N_2 or CO_2) with the electron beam irradiation has the effect of controlling or reducing the development of lipid oxidation and of maintaining the pasteurization effect in irradiated ground pork during refrigerated or frozen storage.

Materials and Methods

Preparation of Ground Pork

Fresh pork (boston shoulder) was purchased from a local meat market. Sample pork was obtained before 24 hrs after slaughter and the visible fat and connective tissues were removed and ground. Prepared pork was divided into 2 portions and purged with N_2 or CO_2. Each half portion were subdivided into the small portions which were inserted into the zipper bag (10 × 14 cm) and purged with N_2 or CO_2. The zipper bags containing pork samples were put into a large zipper bag (35×50 cm) afterwards and purged with the gases one more time. The zipper bags were tightly sealed so that there were no air diffusion into the bags. The N_2 or CO_2 purged samples were irradiated with the electron beam dose of 1.5 and 3.0 kGy and further divided into 2 and stored at 4 and −15℃, respectively. Control(neither MAP nor irradiated) and N_2 or CO_2 control (not irradiated but MAP) samples were also prepared and stored at the same conditions and the treatment effects were compared. Microbial growth and the absorbances of 2-thiobarbituric acid reactive substances (TBARS) were measured during storage at 4℃ for 6 days with 2-day increments and during storage at −15℃ with every month up to 3 months.

N_2 and CO_2 Purging

Ground pork was inserted into a small zipper bag (10×14 cm) first and purged with N_2 or CO_2 gases. Gases were flushed into the zipper bag for approximately 30 sec and sealed tightly. Zipper bags containing each sample were put into a large zipper bag (35×50 cm) and the gases were purged for additional 1 min. Each small zipper bag represented one sample and at the respective days of analysis, one small zipper bag was drawn out of the large bag for the measurements. Prepared samples were ready to be irradiated with the electron beam accelerator.

Electron Beam Irradiation

Samples in the zipper bags were irradiated with a commercial high voltage, Cockraft-Walton type of electron beam accelerator(maximum beam energy: 1.0 MeV, Yeungnam Univ.). Irradiation dose were 1.5 and 3.0 kGy and the beam currents were 0.15 and 0.30 mA, respectively. Conveyer speed was controlled at 10 Hz(2.87 cm/s). The time elapsed for scanning the samples was less than 10 sec but it took additional 10 to 15 min for controlling the accelerator system. The samples were kept at refrigeration temperature until being irradiated but during the period for the irradiation, samples were left at room temperature. The temperature change was very minimal and the samples maintained in the refrigeration temperature. After irradiation, the samples were put back on the ice bath and delivered to the laboratory for the further analyses. Samples were stored at either 4 or −15℃ and the analyses were carried out at every other days and every month, respectively.

Microbial Analysis

Treatments were as followed: ① control, ② N_2 or CO_2 control, ③ modified atomsphere packaged(MAP) and 1.5 kGy irradiated ④ MAP and 3.0 kGy irradiated) and they were either refrigerated(4℃) or frozen(−15℃) and the microbial analyses were made at the following periods: 0, 2, 4 and 6

days for refrigerated storage and 0, 1, 2 and 3 months for the frozen storage.

Total aerobic plate counts were measured according to the procedure of Korean Food Code (2002) using standard plate count agar (Difco, Detroit, MI). Mesophilic and psychrotrophic bacteria were enumerated with the tryptic soy agar (Difco, Detroit, MI). The plates of total aerobic bacteria and mesophiles were incubated at 35 ℃ for 48 hours and psychrotrophs were incubated at 5 ℃ for 48 hrs and counted the colonies. The outline of the microbial analyses were as follows. One gram of each sample was diluted to 100 mL with the sterilized distilled water in a sterilized flask and shaked for 20 min. One milliliter the mixture was picked and diluted to 100,000 times stepwise and innoculated on the petri dish for incubation. Petri dishes containing 30 to 300 colonies were selected and the numbers were counted. All the data were calculated average values for two replicates.

Measurement of Lipid Oxidation

Lipid oxidation was measured with the modified procedure of a distillation method (Tarladgis et al., 1960) and reported as absorbances of 2-thiobarbituric acid reactive substances (TBARS). Five grams of ground pork was homogenized with distilled water and pH value was adjusted to 1.5 with the addition of 4 N HCl. Homogenates were boiled and distilled in the heating block. When the distillates were condensed in the connected condenser, 50 mL of the condensates were collected. Five mL of the condensed distillates were reacted with TBA in the boiling water bath for 35 min and the absorbances of the developed pink color were measured at 532 nm with the spectrophotometer(Uvicon 922, Kontron Instrument, Italy).

Statistical Analyses

All measured values were analyzed with the analysis of variance using SAS program(1996) and the significance of differences among mean values were analyzed with the Student-Newman-Keuls test.

Results and Discussions

Combination Effect of N_2 Purging and Electron Beam Irradiation on the Growth of Microorganisms during Storage

When the ground pork was purged with nitrogen gas (N_2) and irradiated with electron beam, the growth of total aerobic and mesophilic bacteria also increased with increased storage time at 4℃ up to 6 days (Table 1). The control ground pork (neither N_2 purged nor irradiated) had more than 7 log CFU/g of total aerobic bacteria (TAB) and mesophiles after 4 days of refrigerated storage. As shown in Table 1, when the oxygen (O_2) in the air was excluded, N_2 purging alone had an inhibition effect of growth of TAB and mesophiles. In addition, when the samples were N_2 purged in combination with irradiated with electron beam, the growth of both microorganisms were inhibited more than the treatment with N_2 purging alone ($P<0.05$). The order of growth of microorganisms were control, N_2 control, 1.5 kGy and 3.0 kGy. However, no differences in microbial counts were observed with increased dose of irradiation (Table 1). All the microbial

Table 1. Microbial numbers[1)] of ground pork during storage at 4℃ after combination of N_2 purging and electron beam irradiation

Microbial groups	Treatments	Storage time (days)			
		0	2	4	6
Total aerobes	Control	5.36±0.09	5.46^{a}±0.31	7.11^{a}±0.27	7.68^{a}±0.20
	N_2 + Control		5.17^{a}±0.25	6.50^{b}±0.25	6.83^{b}±0.18
	N_2 + 1.5 kGy		4.50^{b}±0.21	4.48^{c}±0.42	5.34^{c}±0.51
	N_2 + 3.0 kGy		3.08^{c}±0.29	4.30^{c}±0.41	5.08^{c}±0.37
Mesophiles	Control	5.34±0.23	5.60^{a}±0.17	7.36^{a}±0.24	7.60^{a}±0.30
	N_2 + Control		5.70^{a}±0.23	6.43^{b}±0.31	6.64^{b}±0.24
	N_2 + 1.5 kGy		3.00^{b}±0.36	4.48^{c}±0.29	5.15^{c}±0.27
	N_2 + 3,0 kGy		3.00^{b}±0.40	4.26^{c}±0.35	5.00^{c}±0.36

[1)] Log colony forming units (CFU)/g. All the values are means of two replicates±standard deviations.
[a-c] Means in the same column having different superscripts are different ($p<0.05$).

Table 2. Microbial numbers[1] of ground pork during storage at −15℃ after combination of N_2 purging and electron beam irradiation

Microbial groups	Treatments	Storage time (months)			
		0	1	2	3
Total aerobes	Control	5.36±0.09	5.40^a±0.41	5.42^a±0.35	5.43^a±0.36
	N_2 + Control		5.35^a±0.27	5.30^a±0.19	5.37^a±0.19
	N_2 + 1.5 kGy		3.78^b±0.31	4.72^b±0.23	4.96^b±0.20
	N_2 + 3.0 kGy		3.23^c±0.24	4.00^c±0.18	4.93^b±0.21
Mesophiles	Control	5.34±0.23	5.36^a±0.19	5.30^a±0.17	5.88^a±0.31
	N_2 + Control		5.37^a±0.27	5.39^a±0.19	5.56^a±0.30
	N_2 + 1.5 kGy		3.04^b±0.40	4.01^b±0.29	4.85^b±0.27
	N_2 + 3.0 kGy		3.08^b±0.31	4.00^b±0.27	4.00^c±0.34

[1] Log CFU/g. All the values are means of two replicates±standard deviations.
[a-c] Means in the same column bearing different superscripts are different ($P<0.05$).

counts of irradiated ground pork were even lower than those of the initial storage (day 0) of control ground pork. This result confirms the pasteurization effect of electron beam irradiation reported by previous studies (Bagorogoza et al., 2001; Farkas, 1998; Johnson and Marcott, 1999; Kim et al., 1998; Kwon et al., 2001; Lee et al., 1998; Olson, 1998; Shamsuzzanman et al., 1995; Thayer and Rajkowski, 1999; 이, 1995). The growth of psychrotrophs was not detected through the storage time (data not shown).

As we expected, the microbial counts for TAB and mesophiles of frozen storage were lower ($P<0.05$) than those of refrigerated storage(Table 2). During 3 months of storage, the increase in microbial growth was slower than that in the refrigerated storage. The irradiation had inhibitory effect ($P<0.05$) for the growth of both microorganisms during frozen storage (Table 2). These results indicated that irradiation (1.5 or 3.0 kGy) was more potent than N_2 purging in terms of microbial inhibition.

Combination Effect of N_2 Purging and Electron Beam Irradiation on the Development of Lipid Oxidation during Storage

When the ground pork was refrigerated after N_2 purging and electron beam irradiation, the values of TBARS tended to be increased with increased storage time (Table 3). Electron beam irradiation was reported to accelerate lipid oxidation (Ahn et al., 1998a, 1998b; Ahn et al., 1999; Du et al., 2001; Hansen et al., 1987; Heath et al., 1990; Hwang and Maerker, 1993; Nam et al, 2001; 이, 1995). But, the absorbance values shown in Table 3 clearly explained that N_2 purging reduced the development of lipid oxidation due to irradiation because of absence of oxygen. The absorbance values of TBARS of 1.5 and 3.0 kGy irradiated ground pork were lower ($P<0.05$) than the control (neither N_2 purged nor irradiated) ground pork. This result showed that the development of lipid oxidation due to electron beam irradiation can be decreased by N_2 purging. In addition, nitrogen packaging combined with

Table 3. 2-Thiobarbituric acid reactive substances (TBARS) absorbances[1] of ground pork during storage at 4℃ after combination of N_2 purging and electron beam irradiation

Treatments	Storage time (days)			
	0	2	4	6
Control	0.0130±0.0010	0.1180^a±0.0017	0.2101^a±0.0016	0.2234^a±0.0016
N_2 + Control		0.0333^c±0.0029	0.0735^c±0.0019	0.1743^a±0.0013
N_2 + 1.5 kGy		0.0481^b±0.0035	0.1011^b±0.0048	0.1215^b±0.0027
N_2 + 3.0 kGy		0.0499^b±0.0015	0.0756^c±0.0049	0.0932^c±0.0020

[1] Means±Standard deviations. All the values are means of two replicates.
[a-c] Means in the same column having different superscripts are different ($p<0.05$).

Table 4. 2-Thiobarbituric acid reactive substances (TBARS) absorbances[1)] of ground pork during storage at −15℃ after combination of N_2 purging and electron beam irradiation

Treatments	Storage time (month)			
	0	1	2	3
Control	0.0130±0.0010	0.2290[a]±0.0030	0.5670[a] ±0.0051	0.4937[a]±0.0040
N_2 + Control		0.2264[a]±0.0017	0.3325[bc]±0.0036	0.3448[b]±0.0029
N_2 + 1.5 kGy		0.1168[b]±0.0024	0.3516[b] ±0.0021	0.3269[b]±0.0036
N_2 + 3.0 kGy		0.1164[b]±0.0031	0.3064[c] ±0.0009	0.2296[c]±0.0015

[1)] Means±Standard deviations. All the values are means of two replicates.
[a-c] Means in the same column having different superscripts are different ($p<0.05$).

electron beam irradiation of ground pork was an effective method to inhibit the growth of microorganisms and to control the development of lipid oxidation in ground pork during storage.

During frozen storage, absorbance values of TBARS also increased with increased storage time up to 2 months (Table 4). As shown in the results from refrigerated storage, N_2 purging had inhibitory effect of reducing the development of lipid oxidation induced by irradiation after 3 month of frozen storage. The absorbance values of TBARS for treatments irradiated with 1.5 kGy and 3.0 kGy were lower ($P<0.05$) than those for control ground pork. These results confirms that N_2 packaging was an effective method to inhibit the development of lipid oxidation induced by electron beam irradiation.

Combination Effect of CO_2 Purging and Electron Beam Iradiation on the Growth of Microorganisms during Storage

Ground pork was refrigerated after purging with carbon dioxide (CO_2) and electron beam irradiation. As the refrigeration periods increased up to 6 days, total aerobic and mesophilic bacterial counts also increased (Table 5). Carbon dioxide purging alone had effects in the inhibition of growth of mesophiles except for 4 days of storage time. As shown in Table 5, both microbial counts were lower ($P<0.05$) in the 1.5 and 3.0 kGy irradiated ground pork than those of control ground pork. Thus, the pasteurization effect of electron beam irradiation was confirmed. It was known that irradiation damaged the DNA structure of bacteria (Bagorogoza et al., 2001). The detection of psychrotrophs was not noted throughout the storage time (data not shown).

In the frozen storage up to 3 months, the numbers of TAB and mesophiles increased with increased storage time, but in a lesser degree than those in the refrigerated storage (Table 6). Electron beam irradiated (1.5, 3.0 kGy) ground pork, in

Table 5. Microbial numbers[1)] of ground pork during storage at 4℃ after combination of CO_2 purging and electron beam irradiation

Microbial groups	Treatments	Storage time (days)			
		0	2	4	6
Total aerobes	Control	4.60±0.16	5.18[a]±0.19	5.95[a]±0.50	6.52[a]±0.41
	CO_2 + Control		5.30[a]±0.26	5.71[a]±0.28	5.98[a]±0.37
	CO_2 + 1.5 kGy		4.08[b]±0.31	4.15[b]±0.30	5.26[b]±0.16
	CO_2 + 3.0 kGy		4.0[b] ±0.33	4.0[b] ±0.37	5.41[b] ±0.15
Mesophiles	Control	4.69±0.18	5.66[a]±0.24	5.79[a]±0.44	6.30[a]±0.37
	CO_2 + Control		4.95[b]±0.36	5.43[a]±0.35	5.53[b]±0.36
	CO_2 + 1.5 kGy		4.0[c] ±0.27	4.70[b]±0.19	5.0b[c]±0.41
	CO_2 + 3.0 kGy		4.0[c] ±0.25	4.30[b]±0.16	4.84[c]±0.21

[1)] Log CFU/g. All the values are means of two replicates±standard deviations.
[a-c] Means in the same column having different superscripts are different ($p<0.05$).

Table 6. Microbial numbers[1] of ground pork during storage at −15℃ after combination of CO_2 purging and electron beam irradiation

Microbial groups	Treatments	Storage time (months)			
		0	1	2	3
Total aerobes	Control	4.60±0.16	4.38[a]±0.39	5.13[a]±0.25	5.51[a]±0.39
	CO_2 + Control		4.23[a]±0.30	5.11[a]±0.29	5.43[a]±0.25
	CO_2 + 1.5 kGy		3.86[b]±0.18	4.32[b]±0.32	5.36[a]±0.27
	CO_2 + 3.0 kGy		3.78[b]±0.13	4.11[b]±0.27	5.04[b]±0.14
Mesophiles	Control	4.69±0.18	4.14[a]±0.17	5.14[a]±0.18	5.51[a]±0.14
	CO_2 + Control		4.23[a]±0.21	5.13[a]±0.27	5.48[a]±0.31
	CO_2 + 1.5 kGy		3.83[ab]±0.15	4.98[a]±0.21	5.40[a]±0.19
	CO_2 + 3.0 kGy		3.45[b]±0.30	4.01[b]±0.16	5.08[b]±0.15

[1] Log CFU/g. All the values are means of two replicates±standard deviations.
[a-c] Means in the same column having different superscripts are different ($p<0.05$).

Table 7. 2-Thiobarbituric acid reactive substance (TBARS) absorbances[1] of ground pork during storage at 4℃ after combination of CO_2 purging and electron beam irradiation

Treatments	Days			
	0	2	4	6
Control	0.0220±0.0010	0.0468[a]±0.0017	0.1729[a]±0.0039	0.1749[a]±0.0048
CO_2 + Control		0.0404[b]±0.0020	0.0740[b]±0.0014	0.0832[b]±0.0027
CO_2 + 1.5 kGy		0.0316[c]±0.0036	0.0593[c]±0.0019	0.0824[b]±0.0033
CO_2 + 3.0 kGy		0.0300[c]±0.0041	0.0379[d]±0.0030	0.0421[c]±0.0019

[1] Means±standard deviations. All the values are means of two replicates.
[a-d] Means in the same column having different superscripts are different ($p<0.05$).

Table 8. 2-Thiobarbituric acid reactive substance (TBARS) absorbances[1] of ground pork during storage at −15℃ after combination of CO_2 purging and electron beam irradiation

Treatments	Storage time (months)			
	0	1	2	3
Control	0.0220±0.0010	0.2349[a]±0.0018	0.2180[a]±0.0049	0.2319[a]±0.0069
CO_2 + Control		0.2341[a]±0.0030	0.2347[a]±0.0051	0.2564[a]±0.0065
CO_2 + 1.5 kGy		0.0891[c]±0.0036	0.1307[b]±0.0037	0.2400[a]±0.0051
CO_2 + 3.0 kGy		0.1472[b]±0.0041	0.1981[a]±0.0059	0.1264[b]±0.0028

[1] Means±standard deviations. All the value are means of two replicates.
[a-c] Means in the same column having different superscripts are different ($p<0.05$).

most cases, especially in 3.0 kGy had lower ($P<0.05$) TAB and mesophiles than control ground pork.

Combination Effect of CO_2 Purging and Electron Beam Irradiation on the Development of Lipid Oxidation during Storage

The absorbance values of TBARS also increased with increased storage time (Table 7). This increase was pronounced especially in the control (neither CO_2 purged nor irradiated). Carbon dioxide purging alone had effect ($P<0.05$) in reducing the development of lipid oxidation when compared to control ground pork. Carbon dioxide purging with

various dose of irradiation (1.5, 3.0 kGy) had lower ($P<0.05$) TBARS absorbances than the control or CO_2 control ground pork. These results indicated that like N_2 purging, CO_2 purging also is an effective method to inhibit the promotion of lipid oxidation caused by electron beam irradiation.

During frozen storage, as shown in the Table 8, CO_2 purging combined with irradiation (1.5, 3.0 kGy), in most cases, had lower ($P<0.05$) TBARS absorbances than both control groups. This also indicates that CO_2 purging has the retarding effect for the promotion of lipid oxidation developed by electron beam irradiation.

The pasteurization effect of electron beam irradiation was also confirmed in the ground pork. The growth of TAB and mesophiles was inhibited by irradiation. The most important finding is that N_2 and CO_2 packagings are effective methods to retard the promotion of lipid oxidation cause by electron beam irradiation. Therefore, N_2 or CO_2 packaging in combination of electron beam irradiation was an effective way to inhibit the growth of microorganisms and the lipid oxidation development.

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요 약

분쇄 돈육을 N_2와 CO_2 가스로 공기 조절 포장한 후 전자선으로 조사하여(0, 1.5, 3.0 kGy) 돈육을 저장하면서 미생물의 변화와 함께 공기 조절 포장이 전자선 처리에 의한 지방 산화를 억제하는지 관찰하였다. 분쇄 돈육은 공기 조절 포장과 전자선 처리(1.5와 3.0 kGy) 후 4 ℃에서 6일간 그리고 −15 ℃에서 3달 동안 저장하면서 주기적으로 미생물 검사와 지방의 산패치를 측정하였다. 일반세균과 중온균의 경우 1.5와 3.0 kGy의 전자선 조사량으로 처리하였을 경우 대조구에 비해 그 증식이 현저히 억제되었음을 관찰하였다. 질소와 이산화탄소를 주입한 포장은 그 자체로도 대조구와 비교하였을 때 저장 중 지방의 산화를 억제하는 데에 큰 효과가 있었으며 또한 공기 조절 포장과 전자선 처리의 병용은 저장 중 분쇄 돈육의 미생물 증식과 지방의 산화를 억제하는데 효과적이었다.

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