Effect of High Hydrostatic Pressure on Inactivation of Pathogens Inoculated onto Beef Loin Packaged with Vegetable Oils

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Abstract— **This study examined the efficacy of high hydrostatic pressure (HHP) in sterilization of *Escherichia coli* (7.5 log CFU/g) and *Listeria monocytogenes* (7.6 log CFU/g) inoculated onto beef loin packaged with grape seed or olive oils. The samples were treated with HHP at 300, 450, and 600 MPa and stored for 10 days at 4℃. Beef loin with HHP at 300 and 450 MPa decreased 2 and 3-4 log CFU/g of the tested pathogens, respectively, regardless of storage day ($p < 0.05$). However, grape seed and olive oils reduced the sterilization capacity of HPP at 450 MPa on *E. coli* and *L. monocytogenes* when compared with control ($p < 0.05$). *E. coli* and *L. monocytogenes* were not detected in beef loin treated with HHP at 600 MPa. Results confirm that HHP is an effective technique to improve the safety of beef.**

Keywords— **high hydrostatic pressure, beef, pathogen**

I. INTRODUCTION

Safety of food is practically considered the most important standard of acceptability for consumers. High hydrostatic pressure (HHP) is used in the food industry to improve the safety and extend the shelf-life of food by sterilizing microorganism [1]. HHP is one of the non-thermal methods that can satisfy the consumer's demand without significant changes in quality of food as heat damage. It is able to apply for foods which are packaged, hence less liable to cross-contamination. However, it is recognized to affect the sensory perception of foods because of impact on texture and lipid oxidation [2].

Olive oil has phenolic compounds that may bring protection by antioxidative and anti-microbiological effect [3, 4]. Grape seed oil contains polyphenolic compound that has excellent antimicrobial, antioxidant and anti-inflammatory properties [5].

The purpose of this study was to evaluate the potential of inactivation of *E. coli* and *L. monocytogenes*, inoculated onto beef loin by HHP when it combined with added vegetable oils and stored at 4℃ for 10 days.

II. MATERIALS AND METHODS

A. Preparation of samples

Beef loins, virgin olive oil and grape seed oil were purchased from a local market. The beef loins were trimmed of all visible fat and cut into similar thickness and weight. The beef loins were packed with the olive oil and grape seed oil (10% of beef loin, w/w) respectively. Then samples were vacuum packaged. For the inoculation test, the samples were sterilized by γ irradiation (44 kGy) using a cobalt 60 irradiator (point source, AECL, IR-79, MDS Nordion, Ont., Canada). The irradiator source strength was 320 kCi with a dose rate of 44 kGy/h.

B. Test pathogens and inoculation

E. coli (KCTC 1682) and *L. monocytogenes* (KCTC 3569) were obtained from a Korean collection for type culture (KCTC, Daejeon, Korea). The strains were cultivated at 37℃ for 24 h in a tryptic soy broth (Difco, Laboratories, Sparks, MD, USA). The activated cell cultures were centrifuged at 3000 x g for 10 min at 4℃ refrigerated centrifuge (Vs-5500, Vision Scientific, Co., Seoul, Korea). The pellet was washed twice with sterile saline (0.85%), and it was finally suspended in saline. The viable cell density was approximately 10^7 – 10^8 CFU/mL. The samples were inoculated with a cell suspension (2% of sample weight, w/w). Each sample was resealed and shaken to be homogenized. All samples were stored in a refrigerator at 4℃ and then transported to the HHP facility in a cooler with icepacks for the pressure treatment.

C. High hydrostatic pressure (HHP)

The equipment used in this study included with a pressure vessel submerged in hydrostatic fluid medium (Quintus food processor 6; ABB Autoclave System, Inc., Columbus, OH, USA). The samples were treated at pressure of 300, 450, and 600 MPa for 5 min with the internal temperature of the pressure vessel at 15±3℃. The control group was placed outside of the cooler for the duration of the pressure treatment in order to expose the samples to a similar condition. Following treatment, all samples were stored at 4℃ until required.

D. Statistical analysis

The data was analyzed by the SAS software (Proc GLM, SAS Institute). The general linear model procedure was processed and the Duncan's multiple range test was used to compare the mean value at $P < 0.05$. Mean values and pooled standard error of the mean (SEM) were reported.

III. RESULTS & DISCUSSION

Table 1 shows the changes of populations of *E. coli* in beef loin after HHP and stored for 10 days. The inoculated number was over 7 log CFU/g and there was no change found after 10 days. Approximately 2, 4, and 7 decimal reductions were achieved after HHP at 300, 450, and 600 MPa, respectively. This result indicates that HHP inactivates *E. coli* in beef loin effectively. The similar trends were observed when vegetable oils were added in beef loin (Table 1). However, when 300 and 450 MPa of HHP were treated, the survived number of *E. coli* were slightly higher in beef loin added with grape seed oil and olive oil. It replies there was no synergistic antimicrobial effect by compounds present in both oils. Nevertheless 600 MPa was enough to eliminate the contaminated pathogen.

The effect of HHP on the inactivation of *L. monocytogenes* is shown in Table 2. The overall trend is similar to the result of *E. coli*. However, a slightly higher resistance in inactivation by HHP was observed when compared with *E.coli*. Previous study reported that *L. monocytogenes* was more resistant against environmental stress [6] and it also may be applied in HHP process.

Table 1. Effects of high hydrostatic pressure on *Escherichia coli* inoculated into beef added with grape seed oil and olive oil during storage at 4□ (log CFU/g).

Treatment	Pressure (MPa)	Storage (day)		SEM[2]
		0	10	
Control	0.1	7.31^{a}	7.29^{a}	0.017
	300	5.20^{bx}	4.95^{bcy}	0.037
	450	3.61^{d}	1.14^{ef}	0.864
	600	ND^{e}	ND^{f}	0.000
Grape seed oil	0.1	7.31^{a}	7.33^{a}	0.023
	300	5.40^{b}	5.56^{c}	0.204
	450	4.43^{c}	3.60^{cd}	0.254
	600	ND^{e}	ND^{f}	0.000
Olive oil	0.1	7.39^{a}	7.69^{a}	0.093
	300	5.37^{b}	5.39^{c}	0.111
	450	3.92^{cd}	2.26^{de}	0.866
	600	ND^{e}	ND^{f}	0.000
SEM[1]		0.220	0.473	

ND = not detected
[1]Standaed error of means (n=36), [2](n=6).
[a-f]Different letters within the same column significantly (p<0.05).
[x,y]Different letters within the same row significantly (p<0.05).

Table 2. Effects of high hydrostatic pressure on *Listeria monocytogenes* inoculated into beef added with grape seed oil and olive oil during storage at 4□ (log CFU/g).

Treatment	Pressure (MPa)	Storage (day)		SEM[2]
		0	10	
Control	0.1	7.51^{a}	7.55^{a}	0.029
	300	5.37^{bx}	4.77^{cy}	0.148
	450	3.93^{d}	3.36^{e}	0.358
	600	ND^{e}	ND^{f}	0.000
Grape seed oil	0.1	7.59^{a}	7.39^{a}	0.060
	300	5.33^{by}	5.71^{bx}	0.072
	450	3.84^{d}	3.57^{e}	0.223
	600	ND^{e}	ND^{f}	0.000
Olive oil	0.1	7.46^{a}	7.29^{a}	0.083
	300	5.39^{by}	5.98^{bx}	0.144
	450	4.59^{c}	4.33^{e}	0.193
	600	ND^{e}	ND^{f}	0.000
SEM[1]		0.183	0.110	

ND = not detected
[1]Standaed error of means (n=36), [2](n=6).
[a-f]Different letters within the same column significantly (p<0.05).
[x,y]Different letters within the same row significantly (p<0.05).

IV. CONCLUSION

High hydrostatic pressure effectively inactivated *E. coli* and *L. monocytogenes* inoculated on beef loin and it must be a good method to improve the safety of beef loin. Addition of vegetable oil for quality enhancement did not affect the inactivation effect significantly.

V. REFERENCES

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