EFFECT OF HIGH HYDROSTATIC PRESSURE ON MICROBIAL REDUCTION AND FATTY ACID COMPOSITION OF BEEF LOIN PACKAGED WITH VEGETABLE OILS

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***Abstract* – This study was conducted to investigate the effect of high hydrostatic pressure (HHP) on microbial reduction and fatty acid composition of the beef packaged with vegetable oils. Twenty four beef loins were vacuumed packed with olive or grape seed oils and were a subject of 2 treatments: no pressure vs 600 MPa pressure. Microbial and chemical analyses were performed on samples stored for 10 days at 4℃. The initial microbial population was approximately 3-4 log CFU/g and increased to 6-7 log CFU/g during storage regardless of treatments. However, HHP with 600 MPa decreased the microbial population to undetectable levels which was also effective at the day 10 of storage. The beef with olive and grape seed oils pressurized to 600 MPa showed significantly higher oleic and linoleic acid contents when compared with the control, respectively, however, it was not the case in the not pressurized samples. The fatty acid composition between the samples with different oil treatments also differed with applied pressure. Results indicate that HHP can improve the safety of beef by reducing microbial population. Furthermore, HHP helped to infuse vegetable oils into beef and altered fatty acid profiles. This technology can be applicable to manufacture value-added meat products for consumers who seek both flavorful and healthy products.**

***Indext Terms*: beef loin, high hydrostatic pressure, vegetable oil, fatty acid composition**

I. INTRODUCTION

The consumers' demand for fresh, safe, and high quality meat products has been increasing. Among the quality factors of beef, intramuscular fat content is one of the main criteria of its evaluation. Marbling can be defined as the appearance of white flecks or streaks of fatty tissue that spreads alongside within the muscle, and is located between the muscle fibers (Harper and Pethick, 2001). Marbling also corresponds to a better eating experience by enriching flavor, improving tenderness and producing more juicy meat (Jo et al., 2010).

High hydrostatic pressure (HHP) technology has been commonly used in the food industry to improve safety and quality of ham, milk, orange juice, and seafood products (Zhang & Mittal, 2008). It can be applied to products which have been already pasteurized and packaged, therefore less susceptible to cross-contamination. However, it is known that HHP can affect some sensory characteristics of foods (Kruk et al., 2009). Furthermore, consumer acceptance of HHP is relatively higher when compared with other non-thermal treatments (Kim et al., 2007). However, the study on improvement of meat quality using the HHP technology is still very limited.

Adding olive and other vegetable oils has been known to improve the quality of processed meat products (Bloukas & Paneras, 1993; Bloukas et al., 1997). Olive oil contains high content of monounsaturated fatty acids (MUFA), especially oleic acid. It is also rich in tocopherols and phenolics that act as antioxidants. Grape seed oil has been also commonly used for both, culinary and pharmaceutical purposes (Bail et al., 2008). Unrefined grape seed oil contains bioactive phenolic compounds that can contribute to beneficial antioxidative effects of products used for human consumption.

Therefore, we have hypothesized that packaging meat, especially lower quality, with vegetable oils and treated with HHP, not only inactivates microorganisms but produces favorable products for human consumption that are, healthier due to the modified fatty acid profiles. Therefore, the objective of this study was to investigate the effect of high hydrostatic pressure on microbial reduction and fatty acid composition of beef loins packaged with vegetable oils.

II. MATERIALS AND METHODS

A. Sample preparation

Twenty four beef loins with similar weight were divided into 2 groups, one treated with a 600 MPa HHP and another not a subject to HHP (0.1 MPa). Beef loins (grade 2), virgin olive oil, and grape seed oil were purchased from the local market. Olive oil and grape seed oil was added (10% of meat weight, w/w) into sample pack and vacuum packaged. The samples without oil addition were considered as controls. All

samples were stored in a refrigerator (4℃) for 6 hrs and then transported to the HHP facility in a cooler on ice for the pressure treatment.

B. **High hydrostatic pressure (HPP)**

The samples were placed in a pressure vessel submerged in hydrostatic fluid medium (Quintus food processor 6; ABB Autoclave System, Inc., Columbus, OH, USA) and pressurized at 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 exposed the samples to a similar temperature. After the HHP, samples were unpacked and excess oils on the surface of the beef was wiped out with the tissue, and samples were repackaged into new bags, vacuumed and stored in the refrigerator until required.

C. **Microbial analysis**

Samples were blended for 2 min with sterile saline using a stomacher. A series of decimal dilutions were prepared and each diluent (0.1 mL) was spread in triplicate on tryptic soy agar (Difco Laboratories, Detroit, MI, USA). The plates were incubated at 37℃ for 48 hr and microbial count was performed. The results were expressed as log CFU/g.

D. **Fatty acid composition and lipid oxidation**

Fat was extracted from the samples according to the method described by Folch et al. (1957). The extracted fat was methylated by adding BF_3-methanol (Sigma-Aldrich Co., St. Louis, MO, USA) in 70℃ water bath for 30 min. The samples were then removed from the water bath, allowed to cool, and 2 mL of hexane and 5 mL of distilled water were added. The upper layer was removed by aspiration and the fatty acid methylesters dissolved in hexane were transferred to a GC vial and analyzed using a GC (6890, Agilent Technologies, Inc., Santa Clara, CA, USA). The GC was equipped with a capillary column (Omegawax 320, 30 m x 0.32 mm x 0.25 μm film thickness, Supelco Inc., Bellefonte, PA, USA) and a flame ionization detector. The temperature of the oven, inlet and detector were 200, 250, and 260℃, respectively. N_2 (99.999%) was used as a carrier gas at a constant flow rate of 0.79 mL/min and a split ratio of 100:1. Fatty acids were identified by comparison of the retention times against the known standards. The relative quantities were expressed as the weight percent of total fatty acids.

Development of lipid oxidation was measured by 2-thiobarbituric acid reactive substances value (Jo et al., 2001).

E. **Sensory evaluation**

Semi-trained panelists (n=10) were used to evaluate sensory properties of the samples. The samples were cooked to the internal temperature of approximately 72℃ using an electronic grill and served to the panelists. A 9-point hedonic scale was used to evaluate the likeness of the product according with the following criteria: liked very much (9), neither like nor dislike (5), and dislike extremely (1). The other sensory parameters evaluated were: color, odor, tenderness, juiciness, chewiness, flavor, overall acceptance, and willingness to buy.

F. **Statistical analysis**

The data was analyzed using general linear models (Proc GLM, SAS Institute, 1989). When there was any significance in ANOVA, a Duncan's multiple range test was applied for the comparison of mean values. Mean values and standard error of the means were reported.

III. RESULTS AND DISCUSSION

The beef loin initially had a microbial population of 3.54 log CFU/g and the addition of vegetable oil increased the population to 3.90 log CFU/g (Table 1). At day 10 of storage at 4℃, the microbial population increased to almost 7 log CFU/g. However, HHP treatment, reduced microbial population below the detection limits (10^2 CFU/g) and maintained it for 10 days in the control and olive oil samples. The beef loins treated with grape seed oil and 600 MPa pressure, increased the microbial count to 2.81 log CFU/g at day 10. The results clearly indicate that HHP treatment is effective in inactivation of microorganisms.

The beef loin packaged with vegetable oils but not pressurized showed no difference in fatty acid composition despite of oil addition (Table 2). Therefore, the addition of oil into the package without pressure treatment could not infuse it into meat. On the other hand, the samples with vegetable oils and pressurized with 600 MPa showed significantly higher contents of oleic acid when packaged with olive oil, and linoleic acid when packaged with grape seed oil, compared to those without pressure treatment. The total unsaturated fatty acids and the ratio of unsaturated/saturated fatty acids were also increased by addition of vegetable oils and the HHP treatment. It is most likely that the pressure applied in the treatment infused the oils into the meat altering its

fatty acid profiles. However, the altered fatty acid profiles with a higher proportion of unsaturated fats increased the rate of lipid oxidation during storage (Data not shown).

There was no significant difference between the treatments in juiciness, flavor, overall acceptance, and willingness to buy the meat (data not shown). However, panelists preferred the color and odor of the beef loin packaged with olive oil and pressurized at 600 MPa. On the other hand, the sample with grape seed oil and pressurized showed significant decrease in color, odor, tenderness, and chewiness compared to the same oil treatment without pressure (data not shown).

IV. CONCLUSION

A combination of vegetable oils and 600 MPa high hydrostatic pressure treatments resulted in beef loins that had more desirable fatty acid profiles, better sensory characteristics and significantly increased shelf-life. Therefore, this method has a potential to become a useful tool in meat processing for manufacturing value-added products appreciated by the consumers.

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Table 1. Microbial population (log CFU/g) of beef with added plant oils and treated with high hydrostatic pressure

Pressure (MPa)	Treatment	Storage (day)		
		0	10	SEM[1]
0.1	Control	3.54^{by}	6.87^{ax}	0.094
	Olive oil	3.90^{ay}	6.71^{ax}	0.129
	Grape seed oil	3.95^{ay}	6.70^{ax}	0.058
600	Control	nd[3,c]	nd^{c}	-
	Olive oil	nd^{c}	nd^{c}	-
	Grape seed oil	nd^{cy}	2.81^{bx}	0.045
	SEM[2]	0.086	0.053	

[1]Standard error of means (n=24), [2](n=8).
[3]Viable cells were not detected within the detection limit of $<10^2$ CFU/g.
[a-c]Values with different superscripts within the same column differ significantly ($p<0.05$).
[x, y]Values with different superscripts within same row differ significantly ($p<0.05$).

Table 2. Fatty acid composition (%) of beef loins packaged with plant oils and treated with high hydrostatic pressure (HHP)

Pressure (MPa)	Oil[1]	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:4}$	SFA[3]	USFA[4]	U/S
0.1	None	25.70	3.63	13.88	42.31	7.25	1.72	0.07	41.90	58.10	1.40
	Olive	25.08^{x}	3.34	13.02	45.74	6.41	1.26	0.08^{y}	40.84	59.16	1.46
	Grape seed	22.43	2.57	13.31	39.79	17.1	0.93^{y}	0.09	37.93	62.07	1.69
	SEM[2]	1.537	0.381	0.967	1.857	3.902	0.221	0.007	2.547	2.548	0.186
600	None	25.90^{a}	3.32^{a}	14.78	41.01^{b}	7.93^{b}	1.45	0.09^{b}	42.80^{a}	57.20^{b}	1.35^{b}
	Olive	21.92$^{b}_{y}$	2.28^{b}	17.33	48.96^{a}	5.12^{b}	1.23	0.15^{ax}	40.99^{a}	59.00^{b}	1.44^{b}
	Grape seed	18.82^{c}	1.75^{c}	10.67	31.93^{c}	31.42^{a}	1.42^{x}	0.09^{b}	30.82^{b}	69.18^{a}	2.29^{a}
	SEM[2]	0.648	0.148	1.770	1.455	2.801	0.164	0.015	2.086	2.086	0.175

[a, b]Values with different superscripts within the same column differ significantly ($p<0.05$).
[x, y]Values with different superscripts within the same column in the same oil treatment differ significantly ($p<0.05$).
[1]1Ratio of samples to oils = 10 % (w/w).
[2]Standard error of the mean (n=9). [3]Saturated fatty acid. [4]Unsaturated fatty acid.