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APPLICATION OF ULTRASOUND AND FRUIT-DERIVED PROTEASES FOR ENHANCING TENDERNESS AND NUTRITIONAL QUALITY OF LOW-GRADE BEEF DURING REFRIGERATED STORAGE

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I. INTRODUCTION

Meat tenderness is a critical determinant of eating quality, substantially affecting consumer preferences and repeat purchasing behavior. However, low-grade beef is often characterized by less tenderness due to its low intramuscular fat content. Thus, various tenderization methods have been explored to enhance its palatability and nutritional profile. Among these, plant-derived proteases such as ficin (*Ficus carica*) extracted from fig and actinidin from kiwifruit (*Actinidia deliciosa*) might be potential due to their proteolytic and antioxidant properties [1]. Moreover, ultrasound treatment has been applied for many foods as a promising non-thermal technology capable disrupting muscle structure, resulting in support enzymatic penetration in the muscle structure [2]. Therefore, this study aimed to evaluate the synergistic effects of combined ultrasound and Fig or kiwifruit juice treatment on the physicochemical, tenderness, and nutritional characteristics of low-grade beef during refrigerated storage.

II. MATERIALS AND METHODS

After external fat and connective tissues were removed, Semitendinosus muscles (Quality Grade 3) from dairy cattle were injected (10%, w/w) with distilled water (CTL), fig juice (FJ), or kiwifruit juice (KJ), with or without ultrasound (US) treatment. The fruit juices were standardized to a proteolytic activity of 10 U/mL. The six treatment groups of CTL, FJ, KJ with or without ultrasound(US)-treated as shown in USCTL, USFJ, and USKJ, respectively. Ultrasound was applied using a 20 kHz, 600 W processor for 30 min (5 min on, 1 min off) in an ice-water bath at 4°C. All samples were vacuum-packaged and stored at 4°C for 0, 7, and 14 days. pH, color (CIE L*, a*, b*), purge and cooking loss, shear force (WBSF), TCA-soluble peptides, myofibrillar fragmentation index (MFI), amino acid and fatty acid profiles, and peroxide value (POV) were measured during storage. SEM was used to evaluate protein degradation and structural changes.-Data were analyzed using two-way ANOVA (treatment × storage time) in SPSS 29.0 ($P<0.05$).

III. RESULTS AND DISCUSSION

There was no significant difference in pH due to ultrasound treatment, but beef injected with KJ showed lower pH values than the control and FJ treatments. For color, L* values were significantly lower in FJ and KJ. At the end of the storage period, both L* and a* values increased, possibly due to improved myoglobin oxygenation and light scattering during blooming. In terms of physicochemical properties, the FJ and KJ-treated groups exhibited lower purge and cooking losses compared to controls, with USFJ and USKJ showing improved water-holding capacity (Table 1). Combining ultrasound and fruit juice treatments significantly improved meat tenderness, indicated by lower

Table 1. Effect of treatments on pH, color, and cooking loss during storage days of raw beef with different pretreatments

Treatment	pH	L*	a*	b*	Purge loss	Cooking loss
CTL	5.55±0.04^{b}	41.70±1.12^{b}	9.95±1.89^{a}	7.14±0.67^{a}	7.33±0.92^{c}	26.59±2.12^{cd}
FJ	5.53±0.10^{b}	38.35±1.73^{a}	9.22±1.47^{a}	6.57±0.63^{a}	2.34±2.09^{a}	25.20±3.26^{bc}
KJ	5.43±0.07^{a}	39.68±1.62^{a}	8.37±1.90^{a}	6.74±1.35^{a}	3.27±2.76^{b}	23.68±6.26^{b}
USCTL	5.56±0.07^{b}	41.31±0.84^{b}	9.42±1.64^{a}	6.44±0.93^{a}	4.70±3.78^{c}	28.20±3.41^{d}
USFJ	5.52±0.12^{b}	38.47±1.69^{a}	9.57±1.06^{a}	6.15±0.63^{a}	2.34±1.90^{a}	23.80±4.73^{b}
USKJ	5.40±0.08^{a}	39.00±0.17^{a}	9.00±2.26^{a}	6.79±1.37^{a}	3.42±2.92^{b}	20.79±7.03^{a}
Storage day						
0	5.52±0.13^{b}	39.01±2.04^{a}	8.45±0.99^{a}	6.56±0.95^{a}	-	28.34±2.99^{b}
7	5.54±0.06^{b}	39.55±2.25^{a}	9.43±1.72^{a}	6.41±1.08^{a}	5.10±0.22^{a}	23.70±5.12^{a}
14	5.43±0.09^{a}	40.70±1.76^{b}	9.89±2.07^{b}	6.94±0.09^{a}	5.27±1.78^{a}	22.10±5.00^{a}

^{a,b}Means with different superscript letters within each column indicate significant differences among treatments or storage days ($p<0.05$). USCTL: Beef was injected with water combined with the application of ultrasonic (19.8 kHz, 600w at 4°C for 25 min); USFJ: Beef was injected with fig juice combined with the application of ultrasonic (19.8 kHz, 600w at 4°C for 25 min); USKJ: Beef was injected with kiwifruit juice combined with the application of ultrasonic (19.8 kHz, 600w at 4°C for 25 min); CTL: Beef was injected with water; FJ: Beef was injected with fig juice; KJ: Beef was injected with kiwifruit juice

WBSF values and increased MFI and TCA-soluble peptides ($p<0.05$) (Figure 1A, B, and C). Among all treatments, USKJ demonstrated the most pronounced tenderizing effect, attributable to the high proteolytic specificity of actinidin for myofibrillar and connective tissue proteins [1]. These findings were also confirmed by microstructural observations from SEM (Figure 3). These results support the synergistic influence of ultrasound and plant proteases on meat tenderization and quality enhancement [3].

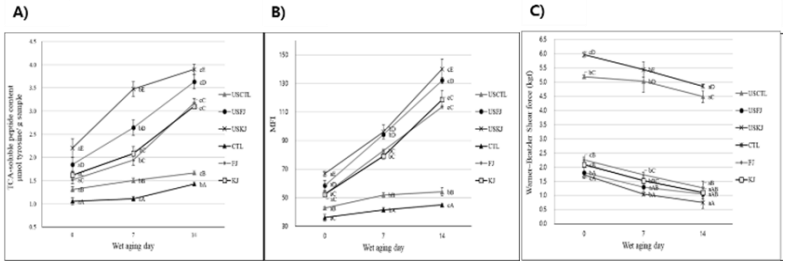


Figure 2 Effect of treatments of TCA-soluble peptide (A), MFI (B), and WBSF (C) during storage days of raw beef with different pretreatments

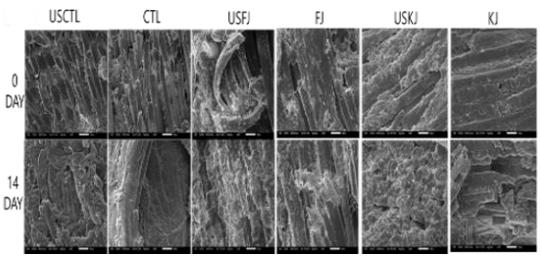


Figure 1 Effect of treatments on SEM during storage days of raw beef with different pretreatments

Ultrasound treatment improved the fatty acid composition of beef. Fig injections alone effectively improved the n-6/n-3 ratio and suppressed lipid oxidation, while kiwifruit showed greater benefits when combined with ultrasound. Combining ultrasound with antioxidant-rich fruit extracts may be an effective strategy to enhance the nutritional quality of beef. However, the effects may vary depending on the type of fruit extract used. While ultrasound treatment proved beneficial in improving the fatty acid profile with kiwifruit, fig extracts appeared to exert their effects without the need for ultrasound treatment, highlighting the importance of tailoring treatment strategies to the specific characteristics of the fruit extracts.

Table 2. Effect of treatments on fatty acid profile during storage days of raw beef with different pretreatments

Treatment	SFA	UFA	MUFA	PUFA	N6	N3	N6/N3
CTL	39.89±1.26^{b}	60.11±1.26^{a}	38.17±3.52^{a}	21.94±2.84^{a}	15.56±1.12^{bc}	5.42±1.79^{a}	3.21±0.91^{b}
FJ	39.50±2.13^{b}	60.50±2.13^{a}	37.87±3.84^{a}	22.63±5.49^{a}	15.49±0.37^{ab}	7.51±0.69^{bc}	1.84±0.85^{a}
KJ	42.39±6.13^{b}	58.23±5.67^{a}	33.40±5.68^{a}	24.82±6.74^{a}	14.19±4.17^{bc}	7.06±3.97^{ab}	3.19±2.11^{b}
USCTL	36.22±1.69^{a}	63.78±1.69^{b}	38.49±6.45^{a}	25.29±5.64^{a}	17.71±5.23^{c}	7.58±1.38^{bc}	2.42±0.74^{ab}
USFJ	41.88±4.20^{b}	58.12±4.20^{a}	35.76±4.70^{a}	22.36±8.23^{a}	11.98±4.77^{ab}	8.99±1.32^{c}	1.47±0.88^{a}
USKJ	41.57±1.49^{b}	58.43±1.49^{a}	36.70±4.67^{a}	21.73±5.65^{a}	11.23±1.84^{a}	6.48±1.41^{ab}	1.79±0.43^{a}
Storage day							
0	39.73±3.64^{a}	60.27±3.64^{a}	37.48±5.25^{a}	22.79±6.59^{a}	12.45±3.10^{a}	7.92±1.97^{b}	1.72±0.78^{a}
14	40.75±3.86^{a}	59.45±3.56^{a}	35.99±4.52^{a}	23.46±4.87^{a}	15.93±4.44^{b}	6.42±2.20^{a}	2.92±1.36^{b}

^{a,b}Means with different superscript letters within each column indicate significant differences among treatments or storage days ($p<0.05$).

CONCLUSION

Enzyme and ultrasound treatment with fig and kiwifruit juice improved the tenderness and nutritional quality of low-grade beef. Using ultrasound further enhanced these effects by promoting enzyme diffusion and proteolysis, improving synergistic efficiency. This combined approach offers an encouraging strategy for upgrading underutilized beef cuts through ultrasound-supported enzyme-based tenderization.

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