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探討在真空滾打下木瓜酵素和鳳梨酵素搭配舒肥烹調法
對改善牛肩肉嫩度之影響

A study on papain and bromelain treatment with
vacuum tumbling under a sous vide cooking for
tenderizing of beef clod

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中文摘要

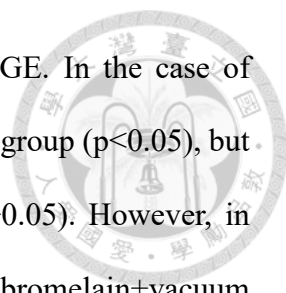
肉是一個優良的蛋白質來源，長久以來一直被人們食用，隨著東亞經濟迅速增長，肉的消費量急劇上升。其中，嫩度對於消費者和烹飪者是一個至關重要的因素。牛肩肉雖營養豐富，然其口感堅韌、烹調後較乾澀，故價格較其他部位肉便宜且多使用於加工上。真空滾打和植物蛋白酶是嫩化肉類的安全有效方法。舒肥法為一低溫長時間烹飪法，不僅改善了嫩度、延長了保存期限，同時提高了食品安全性。儘管真空滾打和植物蛋白酶處理以及舒肥法已經被廣泛研究，然，將真空滾打或植物蛋白酶搭配舒肥處理之研究較為缺乏。故本試驗旨在探討真空滾打和植物蛋白酶對舒肥牛肩肉的協同效應，製備了六組牛肩肉：(1) 控制組牛肩肉 (C)、(2) 經過5分鐘真空滾打的牛肩肉 (CVT)、(3) 加入木瓜蛋白酶的牛肩肉 (P)、(4) 加入木瓜蛋白酶搭配真空滾打的牛肩肉 (PVT)、(5) 加入鳳梨蛋白酶的牛肩肉 (B)、以及(6) 加入鳳梨蛋白酶搭配真空滾打的牛肩肉 (BVT)。首先，比較不同酵素處理的結果，觀察到了嫩度改善、保水性降低和肌原纖維碎片，這與先前的研究結果一致 ($p<0.05$)。此外，還觀察到 L^* 值 (亮度) 的額外增加 ($p<0.05$)。在鳳梨蛋白酶的處理下，硬度明顯降低，比木瓜蛋白酶 ($p<0.05$) 更明顯，而彈性、黏性和咀嚼性也呈現相似趨勢。與木瓜蛋白酶相比，鳳梨蛋白酶的彈性更高 ($p<0.05$)。此外，鳳梨蛋白酶在肌原纖維斷裂指數 (MFI) 方面也表現最高，在 SDS-PAGE 中亦顯示了肌肉重鏈 (Myosin heavy chain, MHC) 和肌動蛋白的減少。在蒸煮損失方面，與控制組相比，鳳梨蛋白酶的蒸煮損失較大 ($p<0.05$)，但與木瓜蛋白酶相比，差異不顯著 ($p>0.05$)。然而，在可擠壓水分 (Expressible Moisture, EM) 方面，鳳梨蛋白酶和鳳梨蛋白酶+真空滾打的處理組之值較其他組高。脂質氧化結果方面，真空滾打處理導致較高的硫代巴比妥酸反應物 (TBARS) 值 ($p<0.05$)。綜上所述，舒肥前的酵素處理對整體質地產生了正面影響，但對保水性產生了負面影響，導致比未經酵素處理的組別更多的水分損失。鳳梨蛋白酶可能是一種酵素，適用於改善牛肩肉等堅硬部位的物理性質。然而，舒肥前的短暫真空滾動似乎不被推薦，因為它對肉質的不良影響，例如增加的 TBARS 值和可擠壓水分。

關鍵字：鳳梨酵素、木瓜酵素、蛋白質降解、嫩度、舒肥、真空滾打

Abstract



Meat is a great protein resource and it has been consumed over a long period. Meat consumption has dramatically risen in East Asia due to rapid economic growth. Tenderness is a crucial factor in meat consumption and in East Asian cuisine. Beef clod is a nutrient dense meat cut, but it is one of the cheapest meat cuts due to its tough texture. Improving the texture of these beef clods can be an excellent way of meat intake in East Asia, where the population is getting old rapidly. Vacuum tumbling and plant protease are safe and effective methods for tenderizing meat. Another culinary technique is sous vide, which involves cooking at low temperatures for a long time. This technique does not only improve tenderness but also extend the storage period and enhances food safety. Although vacuum tumbling and plant protease treatments and sous vide have been studied broadly, the benefits of previous two cooking methods under sous vide has not discussed based on our knowledge. To investigate the synergistic effects of tumbling and plant protease on sous vide-cooked beef clod, six groups of beef clod were prepared: (1) Control Beef clod (C), (2) Beef clod with 5 minutes of vacuum tumbling (CVT), (3) Beef clod with papain tenderizing (P), (4) Beef clod with papain and vacuum tumbling (PVT), (5) Beef clod with bromelain tenderizing (B), and (6) Beef clod with bromelain and vacuum tumbling (BVT). First, for the results of comparing the enzyme treatment, tenderness improvement, water holding capacity decrease, and myofibril fragment were observed as in the previous research results ($p < 0.05$). In addition, in this study, an extra increase for L^* value (Lightness) was seen ($p < 0.05$). In the case of bromelain, the hardness significantly decreased compared to papain ($p < 0.05$), and the similar trend was also shown in cohesiveness, gumminess, and chewiness. Meanwhile, bromelain resulted in higher springiness than papain ($p < 0.05$). Additionally, bromelain also showed the highest value in myofibrillar fragmentation index (MFI) which also echoes that diminished bands of



myosin heavy chain (MHC) and actin were illustrated in SDS-PAGE. In the case of cooking loss, bromelain had higher cooking loss compared to control group ($p<0.05$), but there was no significant difference in comparison with papain ($p>0.05$). However, in terms of expressible moisture (EM), treatments of both bromelain and bromelain+vacuum tumbling cause higher values compared to other groups. Regarding the lipid oxidation of cooked samples, the vacuum tumbling treatment resulted in higher thiobarbituric acid reactive substance (TBARS) values ($p<0.05$). Taken together, enzyme treatment before sous vide cooking positively affected overall texture but negatively impacted on water holding capacity, causing more moisture loss than non-enzyme treated group. Bromelain could be a suitable enzyme applying to improve physical properties of tough beef portion, i.e. beef clod. However, a short period of vacuum tumbling before sous vide cooking doesn't seem recommendable due to its adverse effects on meat quality, such as increased TBARS values and expressible moisture.

Key word: bromelain, papain, protein degradation, tenderness, sous vide, vacuum tumbling

I. Introduction

Meat has been a fundamental component of the human diet for a long time. Beyond its great taste, meat consumption holds significance due to its nutritional value (Abubaker et al., 1996; Williams, 2007). The tenderness of meat is intricately linked to consumer preferences and plays a pivotal role in determining market value (Feuz et al., 2004). Despite a good taste and high nutrient density, certain meat cuts had fallen into disfavor due to their tough texture. In pursuit of improving the overall quality and market value of meat, the meat processing industry is actively engaging in research to explore new technologies and methods for effective tenderization (Bolumar et al., 2013).

Tenderness is a vital component of East Asian cuisine, and meat consumption has significantly increased in East Asia due to rapid economic growth over the last 50 years (Nam et al., 2010). However, this growth was happening at the same time as the super-aged society, marked by a substantial increase in the elderly population in East Asia (Horioka et al., 2018). Elderly people often face difficulties in consuming meat due to poor oral condition and digestive disorders, which may result in malnutrition (Kim, 2021; Liu et al., 2022). This has led to a great demand for elderly-friendly food and a new methods of processing texture-modified meat that with preserving the natural flavor and texture of the original ingredients.

This study is focused on tenderizing beef clod, a cut known for its tough texture due to the heavy connective tissue located between the cattle's shoulders and neck (U.S. Meat Export Federation, 2013). The study employs multiple tenderizing methods, such as vacuum tumbling, sous vide, and plant proteases, specifically Papain and Bromelain. The research aims to compare and analyze the texture characteristics and protein degradation levels under the influence of these tenderizing methods, with the goal of identifying the most effective treatment for enhancing the texture of beef clod.

II. Literature review



2.1. Meat and East Asia

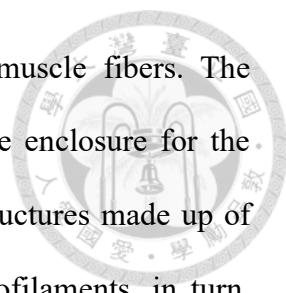
2.1.1. Meat

Meat, known as consumable skeletal muscle, has been a fundamental component of the human diet for a long time. Throughout history, humans have devised various culinary or processing techniques to preserve and process meat, resulting in a wide range of processed meat products (Mann, 2018). The evolution of these processed meat products is influenced by the cultural and climatic characteristics of the regions in which they are created and consumed (Hoogland et al., 2005).

2.1.2. Composition and structure of muscle

A skeletal muscle is composed of four primary elements. The predominant constituent is water, comprising 75%, followed by muscle protein at 20%, fat at 3%, and soluble non-protein substances at 2%. Among the muscle proteins (20%), there are water-soluble sarcoplasmic proteins, such as myoglobins, calpains, and glycolytic enzymes, as well as myofibrillar and connective tissue proteins (Tornberg, 2005). These proteins have different digestibility based on their roles and positions (Sayd et al., 2018). Notably, myofibrillar proteins account for 50% of the total protein in meat. The soluble non-protein substances (2%), consist of 3% minerals and vitamins, 45% non-protein nitrogen substances, 34% carbohydrates, and 18% inorganic compounds (Tornberg, 2005).

The skeletal muscle has an elongated and tubular shape. Meanwhile, its structural sections can be divided by three connective tissue sheaths: Epimysium, Perimysium, and Endomysium. The epimysium surrounds the entire skeletal muscle, serving as a protective enclosure for the contained myofibrils. Each muscle fascicle is wrapped by the



perimysium and consists of cylindrical muscle cells known as muscle fibers. The endomysium surrounds these muscle fibers, serving as a protective enclosure for the contained myofibrils (Park et al., 2022). Myofibrils are intricate structures made up of individual contractile proteins known as myofilaments. These myofilaments, in turn, consist of repeated cylindrical organelles called sarcomeres, recognized as the smallest functional unit of muscle contraction (Lin et al., 2011). The myofilaments can be categorized into actin (Thin) and myosin (Thick) filaments (**Figure 2-1**). The interaction between these two filaments reduces the gap between myofibril, resulting in muscle contraction. Myosin filament is made up of myosin proteins, which connected by branches called cross-bridges. At the end of each branch, there is a round structure resembling bean sprouts called myosin head. Myosin head has binding sites for both actin and ATP, playing an important role in cross-linking with actin. Actin is a structural protein that makes up the thin filaments of myofibril. Together with myosin, it forms the basis of muscle contraction.

Actin is named after its role in activating myosin, which is only able to contract when it coexists with actin. The various names of sarcomere are determined by the brightness difference of the colors observed under a microscope. Each sarcomere is divided into two regions called Z disks (**Figure 2-2**), characterized by a deep darkness. The Z disk is a dense collection of proteins that prevents light to pass through. The region between Z disks is composed of two bright areas called the I band and a darker gray area called A band. The I band appears brighter because it is mainly composed of actin filaments that allow light to pass through. On the other hand, A band is mostly composed of myosin filaments that are longer than actin filaments, making it appear darker. The area adjacent to the I band in the A band contains both actin and myosin filaments. Inside the A band, there is a relatively bright area called the H zone, which is bright because actin and myosin

do not overlap in the relaxed state of the muscle. Finally, the A band is bisected by a dark central line called the M line.

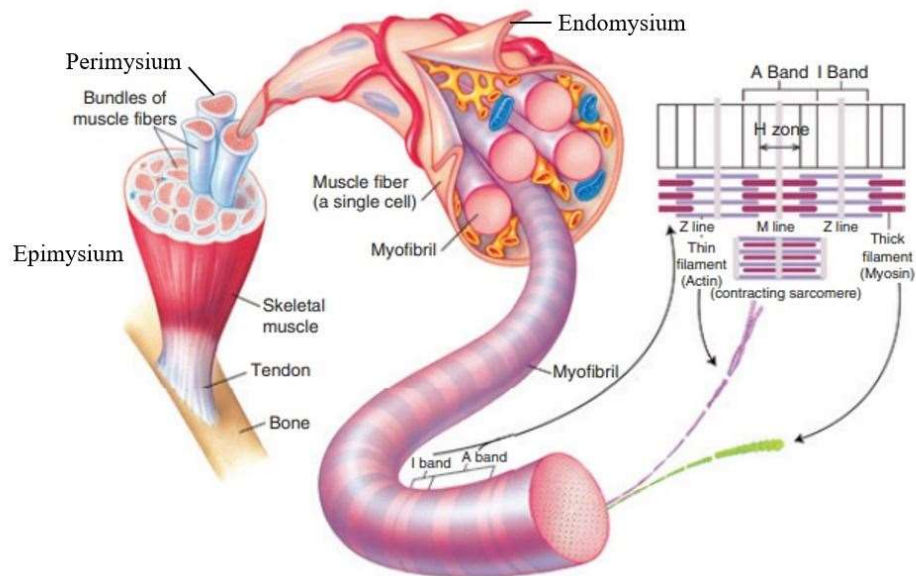


Figure 2-1. Details of the skeletal muscle structure and the contractile machinery of the muscle cell.

(Source: <https://www.pharmacy180.com/article/structure-of-skeletal-muscle-fibers-3522/>)

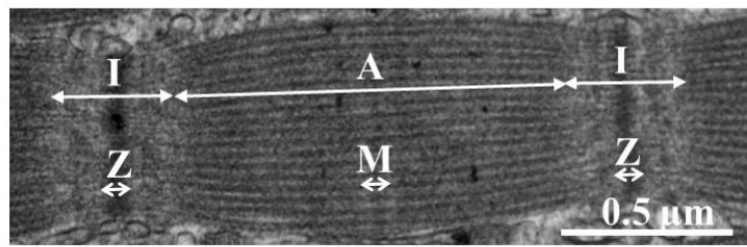
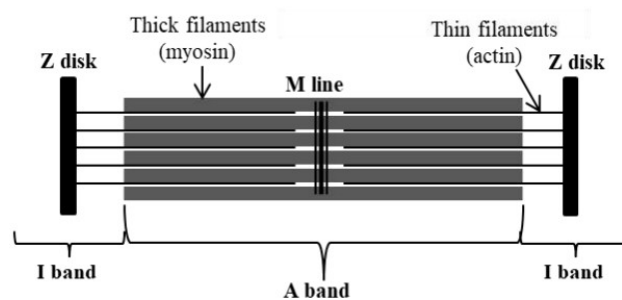


Figure 2-2. Diagram of striated muscle sarcomere.
(Listrat et al., 2016)

2.1.3. Nutrition Value of beef

Cattle (*Bos taurus*) meat, commonly known as beef, is an excellent source of protein, unsaturated fats, minerals, and vitamins that are essential for human health according to studies conducted by Oh et al. (2016). Protein is vital for cell composing in the body and producing enzymes, hormones, and antibodies (Abubaker et al., 1996). Fat provides energy and protects inner organs and nerves from collision, while it assists in absorbing lipid-soluble vitamins A, D, E, and K (Fennema, 1996). In the case of beef, the content of saturated fatty acids and monounsaturated acids is relatively high compared to pork and chicken due to its high fat content. Due to the microorganisms in remen, trans-fatty acids (18:1 *trans*) associated with cardiovascular disease were about 3% of the total fatty acids in beef, higher than 2% of pork and 1.5% of chicken (**Table 1-1**). Vitamin is necessary to maintain the physical function of cells (Pereira & Vicente, 2013), and beef is also a rich source of niacin (Vit B₃) and cobalamin (Vit B₁₂) (**Table 2-2**). It also contains a small amount of vitamin D and encompasses all essential amino acids except tryptophan, making it a great source of protein (Williams, 2007). Additionally, it contains a significant

amount of creatine, which plays a crucial role in muscle energy metabolism by converting into creatinine during the cooking process (Elbir & Oz, 2021).

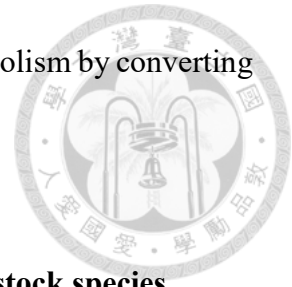


Table 2-1. Amount of the fatty acid in meat (100g) sorted by livestock species.

Fatty acid	Beef (g)	Pork (g)	Chicken (g)
C14:0	0.096	0.010	0.020
C15:0	0.012	0.000	0.000
C16:0	0.607	0.250	0.340
C17:0	0.028	0.000	0.010
C18:0	0.356	0.130	0.120
Total saturated fatty acid	1.149	0.400	0.500
C14:1	0.025	0.000	0.000
C16:1	0.082	0.030	0.004
C18:1	1.103	0.390	0.620
C20:1	0.015	0.010	0.010
Total monounsaturated fatty acid	1.205	0.430	0.700
C18:2 ω -6	0.240	0.120	0.210
C18:3 ω -3	0.048	0.010	0.010
C20:3 ω -6	0.020	0.003	0.008
C20:4 ω -6	0.076	0.019	0.030
C20:5 ω -3 (EPA)	0.031	0.000	0.005
C22:5 ω -3 (DPA)	0.051	0.006	0.009
C22:6 ω -3 (DHA)	0.006	0.004	0.009
Total polyunsaturated fatty acid	0.448	0.200	0.300

* DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid. (Williams, 2007)

Table 2-2. Amount of nutrients and daily value of cooked beef.^{ab}

Nutrient	Amount in beef	% Daily Value	Claim
Vitamin B ₁₂	2.45 µg	102%	Excellent Source
Zinc	8.5 mg	77%	Excellent Source
Selenium	29 µg	53%	Excellent Source
Vitamin B ₃	6.5 mg	41%	Excellent Source
Riboflavin	0.3 mg	23%	Good Source
Pantothenate	1 mg	20%	Good Source
Iron	3.5 mg	19%	Good Source
Phosphorus	200 mg	16%	Good Source
Vitamin B ₆	0.25 mg	15%	Good Source
Choline	70 mg	13%	Source
Thiamin	0.075 mg	6%	Source
Magnesium	25 mg	6%	Source
Potassium	300 mg	6%	Source

* a Calories 250 calories, 35 g protein, 10 g fat, 0 g carbohydrate, 55 mg sodium

** b Per 100g serving

(Beef Cattle Research Council, 2017).

2.1.4. Cuts of beef

Each cut of beef has unique nutritional composition, physico-chemical properties and flavor, leading to varying consumer preferences (Cho et al., 2007). According to Korea Institute for Animal Products Quality Evaluation (KAPE), high valued cuts, such as the loin and tenderloin, are sold at auctions for three times more expensive than the price of less popular cuts like topside and shank (Korea Institute for Animal Products Quality Evaluation, 2023).

The carcass is commonly divided into large cuts called primal cuts, which are further divided into smaller pieces known as sub primal cuts. **Figure 2-3** presents a diagram of the 8 primal beef cuts according to American standards.

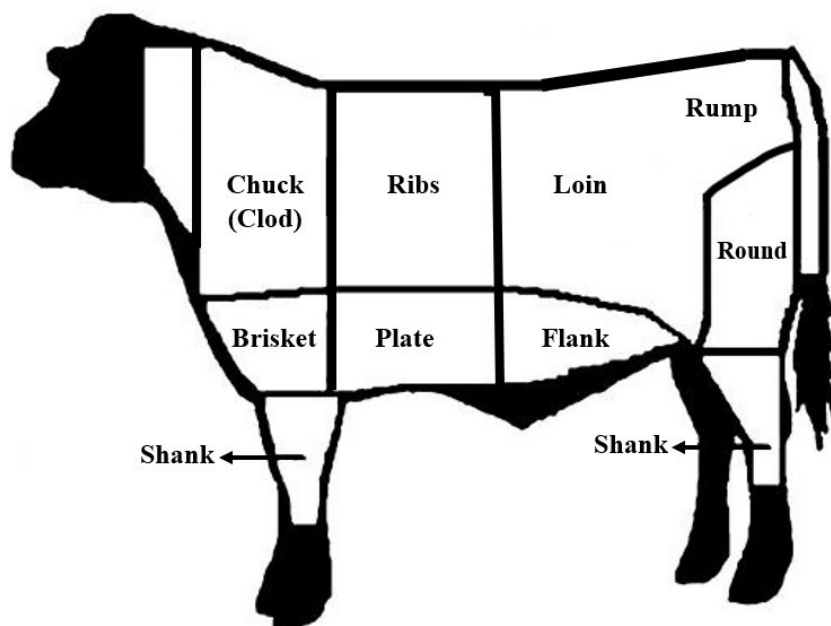
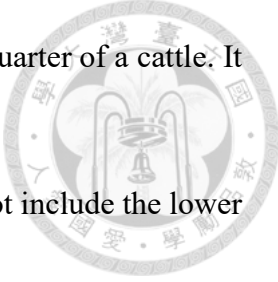


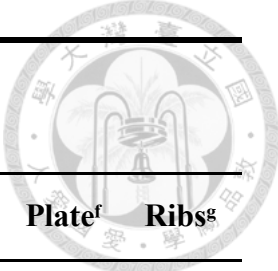
Figure 2-3. Diagram of eight beef primal cuts (American standard).
(Modified from Valenzuela et al., 2009)

- 
- i. Chuck: Beef chuck is a cut of beef that comes from the forequarter of a cattle. It includes the upper arm, shoulder blade, and part of the neck.
 - ii. Ribs: Ribs consist of the 6th through the 12th ribs but does not include the lower portion, also known as the plate.
 - iii. Loin: Loin is located on the top of the cattle, right behind the rib, the loin is the highest-quality cuts that can get from beef.
 - iv. Round: Round is located at the cattle's back, while the rump is at the hind legs.
 - v. Flank: Flank is a boneless cut of meat situated below the loin.
 - vi. Plate: Plate also called the short plate, and is located below the rib cut, in the forequarter of the cattle.
 - vii. Brisket: Brisket is from the cattle's breastbone area and is in the front of the cattle, below the chuck.
 - viii. Shank: Each side of beef has two shanks, one in the forequarter and one in the back. The shank is the upper part of the cattle's legs.

2.1.5. Beef clod

Beef clod is one of the subprime cuts in the chuck, located near the brisket. It is composed of three main muscles: the shoulder tender, the top blade, and the clod heart (U.S. Meat Export Federation, 2013). beef clod is known for its toughness with connective tissue due to heavy workload in life cycle of a cattle, making it one of the cheapest cuts of beef (Silver Fern Farm, 2023). However, it contains a higher amount of zinc and selenium compared to other parts from different cuts (**Table 2-3**). Beef clod is also known for its great taste and is being used in many different cuisines. To make the most of its flavor, beef clod is often cooked by using moist heating, braising methods for stew, and shredding for making hamburger patty. (U.S. Meat Export Federation, 2013).

Table 2-3. Mineral contents of beef products from different cut.



Mineral (mg)	Beef product (100g)	Round ^b	Flank ^c	Brisket ^d	Loin ^e	Plate ^f	Ribs ^g
	Shoulder clod ^a						
Calcium	6	5	24	12	14	6	10
Iron	2.62	2.91	1.54	2.02	2.47	3.63	1.66
Magnesium	23	24	22	22	12	20	17
Phosphorus	198	217	197	206	219	147	117
Potassium	324	360	326	336	295	251	224
Sodium	75	62	54	82	42	63	52
Zinc	7.47	4.31	3.6	5.05	3.33	5.13	4
Copper	0.116	0.132	0.074	0.063	0.067	0.167	0.06
Manganese	0.017	0.02	0.01	0.002	0.008	0.167	-
Selenium (µg)	31.5	30.3	24.5	25.4	21.3	23.1	16.7

* a-g: separable lean and fat, trimmed to 0" fat, select, raw.

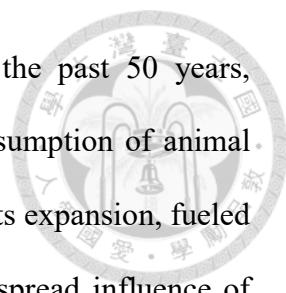
(U.S. Department of Agriculture, 2019)

2.1.6. Characteristics of East Asian food

East Asia and the Western world have different perspectives on food. For example, in China, food is not just a means of obtaining nutrition but also a way to emotionally fulfill oneself. This perspective views food as an art (Kittler & Sucher, 1998). In this manner, it is not surprising that tenderness is one of the most essential parts of Chinese food, as does Japanese Washoku (traditional Japanese cuisine) over all (Nam et al., 2010; Matsuishi, 2022).

2.1.7. Meat consumption in East Asia

It is well known that the Western has historically dominated the meat processing industry due to its environmental and cultural factors. However, the recent economic growth and infusion of Western cuisine have significantly impacted dietary habits and meat consumption trends in the East Asian region (Nam et al., 2010) (**Table 2-4**). Korea



and China have experienced remarkable economic growth over the past 50 years, resulting in a notable increase in livestock production and the consumption of animal products. The meat processing industry in East Asia is prepared for its expansion, fueled by the rapid economic growth in East Asian nations and the widespread influence of Western culture (Zhang et al., 2017). Furthermore, the OECD-FAO Outlook 2019 (Meat & Livestock Australia., 2020) predicts a significantly increased beef consumption across the entire Asia region, projecting a growth of 2.7 million tons in carcass weight equivalent (cwe) over the next decade. This increased demand for meat, especially beef, is anticipated to be driven by the rising incomes in developing countries in Southern east Asia. Notably, this surge in consumption in Asia outpaces that of any other region globally (**Figure 2-4**). Meat and meat-related products market in East Asia is expected to grow continuously. However, to sustain this growth, consistent investment is required from governmental policy, industry, and educational institutions (Nam et al., 2010).

Table 2-4. Changes of per capita income, number of major species of animals in Korea and China.

	Year			Increase rate (1956–2006)
	1956	1985	2006	
Korea				
Per capita income (US\$)	66	2,309	17,500	265.1
Cattle number (MH)	0.87	2.94	2.48	2.8
Pig number (MH)	1.26	2.85	9.38	7.4
Chicken number (MH)	8.92	51.08	119.18	13.3
Consumption of animal products (kg/person/year) ^a	6.10	42.20	105.33	17.2
China				
Per capita income (US\$)	59	292	2,034 ^c	34.5 ^c
Cattle number (MH)	49.51	62.71	117.66	2.3
Pig number (MH)	85.62	313.86	510.62	6.0
Chicken number (MH)	540.85	1,266.89	4,356.97	8.1
Consumption of animal products (kg/person/year)	8.40	28.50	92.94	11.1

* a Data including beef, pork, chicken meat, eggs and milk.

** Data adjusted from on-line information (www.nationmaster.com).

(Nam et al., 2010)

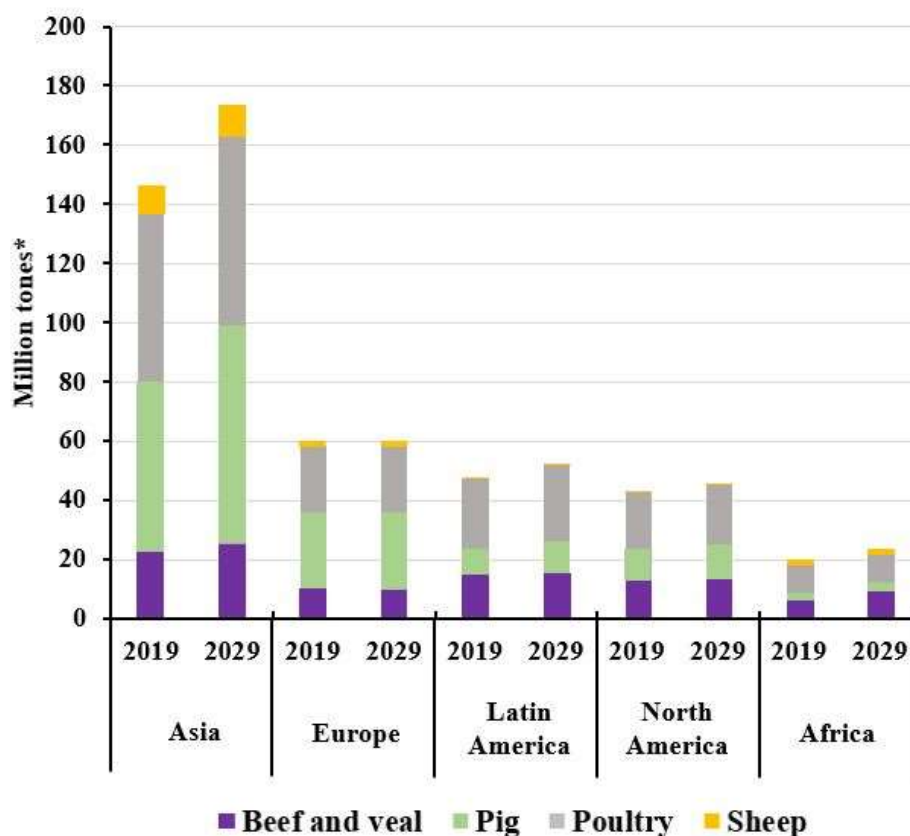


Figure 2-4. Global meat consumption of 2019 and 2029 (anticipation) sorted by livestock species.

* In million tonnes of carcass weight equivalent (cwe).

(Meat & Livestock Australia., 2020)

2.1.8. Super-aged society in East Asia

According to United Nations (UN), a country is considered an aging society when more than 7% of its total population is aged 65 or older. If this portion reaches over 14%, it is classified as an aged society, and if it reaches over 20%, it is called a super-aged society. East Asia is currently the fourth-largest population of people aged 65 or older so far. However, this number is expected to increase significantly by 2065, leading to the highest ratio of elderly people in the world. Moreover, the increasing rate of the elderly population in East Asia is rising the most dramatically compared to other continents (Table 2-5).

Table 2-5. The share of the population age 65 or older in the total population by region (%).

Region	2015	2065 (projected)	Change (2015-2065)
World	8.3	18.3	10.0
Africa	3.5	7.9	4.4
Europe	17.6	28.5	10.9
Latin America	7.6	24.9	17.3
North America	14.8	24.7	9.9
Oceania	11.9	20.2	8.3
Asia	7.6	21.7	14.1
East Asia	11.1	30.9	19.8
Central Asia	4.8	16.4	11.6
South Asia	5.4	18.1	12.7
Southeast Asia	5.9	19.0	13.1
West Asia	5.2	16.9	11.7

* These shares are weighted by population. East Asia includes China, Hong Kong SAR, Japan, Macao SAR, Mongolia, North Korea, South Korea, and Taiwan.

** Data adjusted from United Nations, 2017 Revision of World Population Prospects (<https://esa.un.org/unpd/wpp>).
(Horioka et al., 2018)

Elderly people often have problems with dental and gum issues, as well as digestive disorders due to reduced secretion of digestive enzymes. This can lead to nutritional imbalance and malnutrition (Kim, 2021). In light of these challenges, the concept of elderly-friendly food has emerged. Elderly-friendly food is food manufactured and processed by modifying its physical traits to aid consumption and digestion for elders (Lee et al., 2020). As the baby boomer generation with the economic influence with wealth ages, the influence of elderly people in the consumer market is expected to increase both quantitatively and qualitatively (Kim, 2021). A survey conducted among individuals over 65 years old revealed that 52.6% of the respondents considered elderly-friendly food ‘necessary,’ while 25.1% considered it ‘very necessary.’ This indicates a gradual increase in demand for elderly-friendly food (Kim et al, 2017; An et al., 2018).

Japan has been classified as a ‘super-aged society’ since 2006, with the proportion of individuals over 65 exceeding 20%. With the rapid increase in Japan's elderly population, the number of individuals requiring nursing care has also seen a significant rise, emerging as a notable societal concern (Horioka et al., 2018). Regarding this issue, the Ministry of Agriculture, Forestry, and Fisheries in Japan has implemented policies promoting elderly-friendly foods called ‘Smile Care Food’ since the 2010s. **Figure 2-5** describes the process of selecting suitable smile care food for individuals.

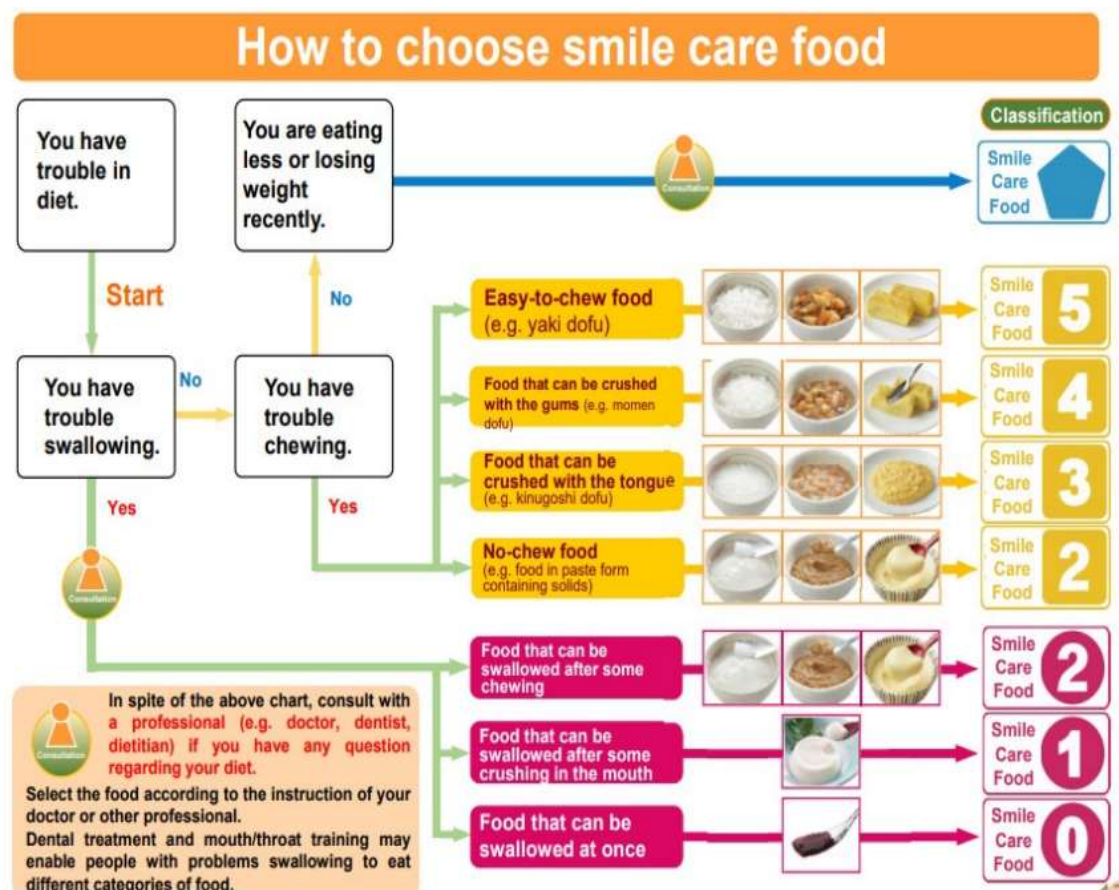


Figure 2-5. Guideline of smile care food classification.

(Source: https://www.maff.go.jp/e/policies/food_ind/attach/pdf/index-9.pdf)

2.2. Meat palatability

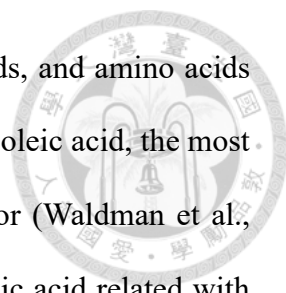
Meat palatability is a crucial factor in determining the overall dining experience and perception of meat as a protein source (Miller, 2014). In fact, palatability is a major reason that affects the amount of food consumed (de Graaf et al., 1999). There are numerous factors that contribute to the palatability of meat, such as lipid and fatty-acid content, portion of lean meat, the presence of connective tissue in muscles etc. Due to its influence on consumer acceptance, meat palatability is regarded as important as its nutritional value. The palatability of meat hinges on three major factors: juiciness, flavor, and tenderness (Miller, 2014). A combination of environmental and nutritional conditions is also related with these three factors. Furthermore, both pre and post-slaughter management and the cooking methods applied before the meat reaches the end consumer can affect the overall palatability of meat.

2.2.1. Juiciness

Juiciness is an indicator of the water content perceived in the mouth during meat consumption. Because of heating, various ingredients preserved in the meat tissue leak out along with moisture and fat. When chewing meat, the juice in the mouth could stimulate the secretion of saliva. Cho (2002) reported that juiciness is affected by the water content and the degree of intramuscular fat in the meat. Juiciness and tenderness are closely related, often noted in the observation that more tender meat tends to release juices more readily, resulting in a perception of greater juiciness.

2.2.2. Flavor

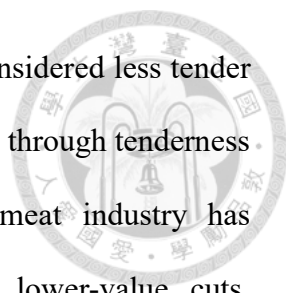
Flavor is a complex sensation that human perceive through a tongue, nose, and the pressure and heat in the mouth. It is mainly determined by nucleic acid-related substances



generated by metabolism, the types of fatty acids that make up lipids, and amino acids that are broken down from proteins (Cho et al., 2007). Beef contains oleic acid, the most abundant fatty acid, and is known to correlate positively with flavor (Waldman et al., 1968; Westering & Hedrick., 1979). The increased proportion of oleic acid related with the increased amount of intramuscular fat content (Sturdivant et al., 1992; Oka et al., 2002). Fresh meat may not have any odor, but cooking brings out the unique flavor out of meat. Even beef with same texture or from the same part of the animal can taste different depending on the cooking method. This is because various types of volatile, complex substances generated by different culinary methods can stimulate the sense of smell and taste, enhancing the flavor (Bakanowski et al., 1984).

2.2.3. Meat Tenderness

Tenderness refers to how easily teeth can penetrate meat when it is being chewed. In other words, it is a measure of how soft the meat is. Feuz et al. (2004) mentioned that tenderness plays an important role in determining the overall quality of meat, and many customers are even willing to pay extra for tender meat. Tenderness is evaluated based on the force required to penetrate meat during chewing, the amount of crushed meat when chewing the meat inside the mouth, and the amount of meat juice that remains in the mouth after chewing (Ouali, 1990). Meanwhile, tenderness depends on factors such as muscle contraction and intramuscular fat content. The elution of muscle fat during meat chewing works as a lubricant, indirectly enhancing the overall palatability and tenderness of the meat (Cho et al., 2007). Connective tissue (collagen) also partially contributes to its variation in meat tenderness (Weston et al., 2002). In general, the proportion of connective tissue tends to increase as animals age, resulting in tougher meat. Therefore, it is necessary to apply long-time cooking methods or techniques such as braising,



casseroling, or pressure cooking to lessen toughness. Like chuck, considered less tender part of beef, can be turned into more affordable and desirable options through tenderness improvement (tenderization) (Von Seggern et al., 2005). The meat industry has consistently created innovations to enhance the tenderness of lower-value cuts. Simultaneously, developed inventions to preserve the high-quality attributes of high-value cuts (Bolumar et al., 2013). However, it should be noted that the tenderness is not entirely isolated from the other two components of palatability mentioned above. Considering the interconnected of palatability factors, a comprehensive analysis of palatability demands a diverse range of perspectives and approaches.

2.2.4. Measurement of palatability

When assessing palatability, there are many different factors contribute to the overall complexity of the measurement process. Furthermore, there are even external factors, such as the type of rheological test, the contraction state, and the cooking procedure, that can also influence the outcome of these assessments (Lepetit, 1989). Thus, diverse measurement techniques have been devised to provide a more comprehensive interpretation and understanding of these concepts. There are four commonly used methods to analyze palatability. The following are the common methods used to analyze palatability:

i) Cooking loss: Nutrients present in foods can leach into water or oil during the cooking process, undergoing breakdown due to heat, or experiencing partial loss. This phenomenon is referred to as cooking loss. The weight lost during cooking can be measured to determine the degree of cooking loss, and this serves as a useful indicator for evaluating the meat juiciness (Gault, 1985; Hui et al., 2012).

ii) Texture profile analysis (TPA): TPA simulates mastication by employing the same principles as human continuous chewing, measuring the physical properties of food, also known as mechanical texture. One of the primary advantages of TPA is its ability to evaluate multiple parameters at the same time, including hardness, springiness, chewiness, gumminess and cohesiveness, all within a single testing procedure (Hui et al., 2012). Figure 2-6 displays a schematic diagram that represents the correlation between force and time and following are the common parameters used to analyze texture:

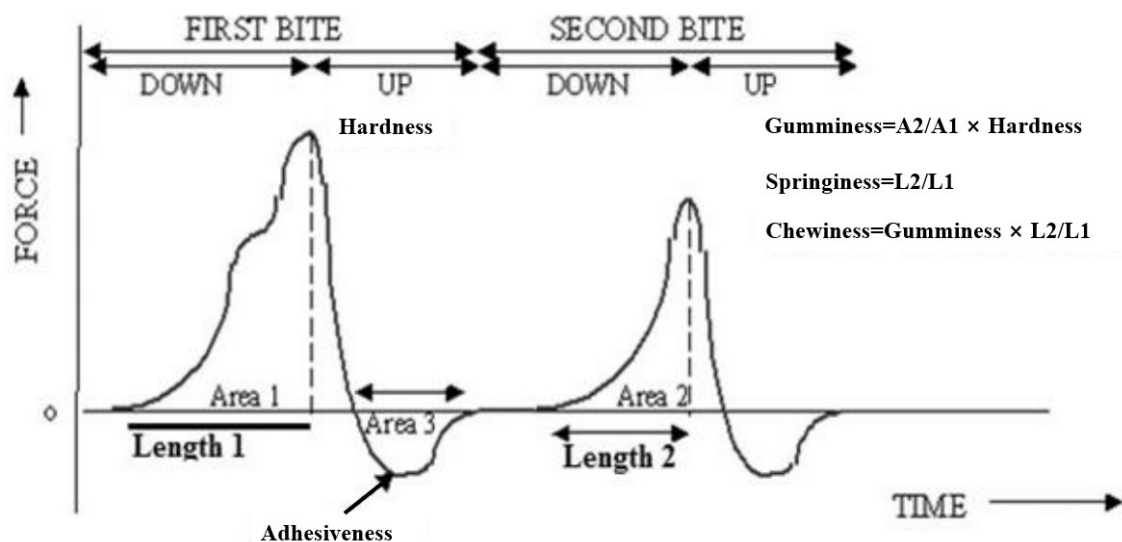


Figure 2-6. Schematic diagram of texture profile analysis calculations

- **Hardness:** Hardness is the measure of a material's resistance to deformation, indicating the strength of internal bonding forces that maintain its structure. Hardness serves as a direct indicator of taste perception that affects texture profile analysis parameters like chewiness, gumminess, and Cohesiveness.
- **Springiness:** Springiness indicates the how high food can rebound between the end

of the first bite and the beginning of the second mouthful.

- **Chewiness:** Chewiness is the energy required to chew solid food and is calculated by the gumminess x springiness. This parameter is challenging to measure accurately due to the complex nature of chewing traits, which involves compression, shearing, puncture, comminution, tearing, cutting, and is influenced by oral conditions such as saliva secretion and body temperature. Chewiness is primarily used to describe solid and semi-solid mouthfeel.

- **Gumminess:** Gumminess is calculated by hardness x cohesiveness. It is an important parameter for describing the taste of semi-solid foods, characterized by low hardness and high cohesion.

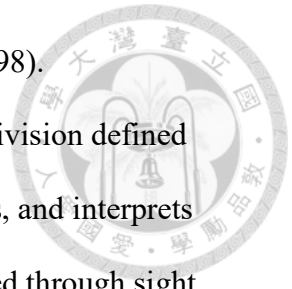
- **Cohesiveness:** Cohesiveness is the ratio of the first compression to the second compression positive force area. If a better sample is condensed, the display probe is also easier to keep clean. Cohesiveness is typically a lesser-tested parameter compared to chewiness, and gumminess.

iii) Warner-Bratzler shear force (WBSF): WBSF is a standard measurement method that can scientifically assay the tenderness that determines the palatability of meat.

Shearing force is defined as the mechanical force used to gauge the tenderness and toughness of meat while it quantifies the amount of force (expressed in Kg/0.5 inch²) required to place meat into a WBSF machine and cut it into a 1/2 to 1-inch thick cylindrical shape. The shear force is determined by measuring the maximum pressure recorded during a test, usually in newtons or kilograms. On the other hand, shear energy is calculated by determining the area under the force deformation curve, extending from the beginning to the end of the test, and is typically measured in newton millimeters (newton-mm) (Hui et al., 2012). The WBS test is also known for demonstrating a strong

correlation with sensory panel scores for meat toughness (Monin, 1998).

iv) Sensory analysis: Institute of Food Technologists (IFT) sensory division defined sensory analysis as a scientific measurement that quantifies, analyzes, and interprets reactions to the sensual characteristics of food and materials perceived through sight, smell, taste, touch, and hearing (Miller, 2023). In this context, a person serves as a measuring instrument, evaluating food quality attributes. Sensory analysis is essential in developing consumer-desired products by analyzing the sensory characteristics of food and understanding its impact on consumer preferences. Sensory tests are broadly categorized into three types. The discriminant test is used to evaluate the variation in taste acceptability among different samples or foods as perceived by a group of panels. Descriptive analysis, on the other hand, helps to identify and describe the differences between samples in detail, while affective tests measure how much the panels like a particular sample or food and which one they prefer over others. Sensory analysis may need some experts, laboratory-trained professionals, or even consumer panels. However, although the sensory analysis is a time-consuming and costly process, using the human five senses is still considered necessary since no laboratory analyses or mechanical devices can accurately replicate human perceptions (Hui et al., 2012). Through this delicate procedure, efficient sensory testing can bring out the potential to improve product development and quality control, resulting in increased company growth through financial benefits and time-saving.



2.3. Meat tenderizing



Tenderization is a process aimed at improving the tenderness of meat while it helps to maximize the potential tenderness from tougher cuts, offering an optimal texture at a reasonable cost, thus benefiting both producers and consumers. Beyond its impact on palatability, meat is a rich protein source. Therefore, tenderization is particularly important for those who have difficulty with chewing and swallowing, and people dealing with digestion disorders in terms of adequate protein intake for malnutrition prevention (Liu et al., 2022). There are various ways of improving the tenderness of meat, including mechanical, chemical, and enzymatic (plant proteases) tenderness (IGNOU, 2017).

2.3.1. Mechanical tenderization

Mechanical tenderization is a method of improving tenderness by applying physical force on the meat. This method utilizes equipment such as needles, blades, tumblers, and grinders to exert force directly on the muscle fibers. The physical force disrupts collagen bonds and breaks down muscle fibers, thereby improving the efficiency of proteolytic enzymes during the cooking process (IGNOU, 2017). Mechanical tenderization has a profound impact on the cellular structure of meat. This activation facilitates the autolysis process in meat, ultimately improving tenderness and increasing protein digestibility.

2.3.2. Vacuum tumbling

Vacuum tumbling is a widely adopted mechanical tenderizing method using impact energy from forcing meat pieces to fall from the top of a rotating drum. **Figure 2-5** describes the visual of vacuum tumbler, the drum equids segmented baffles inside that, upon rotation, gently rub and knead the meat (**Figure 2-5**). Through the constant flipping

and colliding within the tumbler, muscle cells undergo rupture, creating pathways for marinades. It promotes the absorption of the marinade into the meat, thereby shorting marinating time (IGNOU, 2017). The vacuum conditions in tumbling prevents the creation of bubbles after tumbling, thereby making exudates present on the surfaces of meat proteins. Additionally, the vacuum condition contributes to meat tenderizing, reducing cooking losses and preventing water loss in meat (Chouljenko et al., 2017).

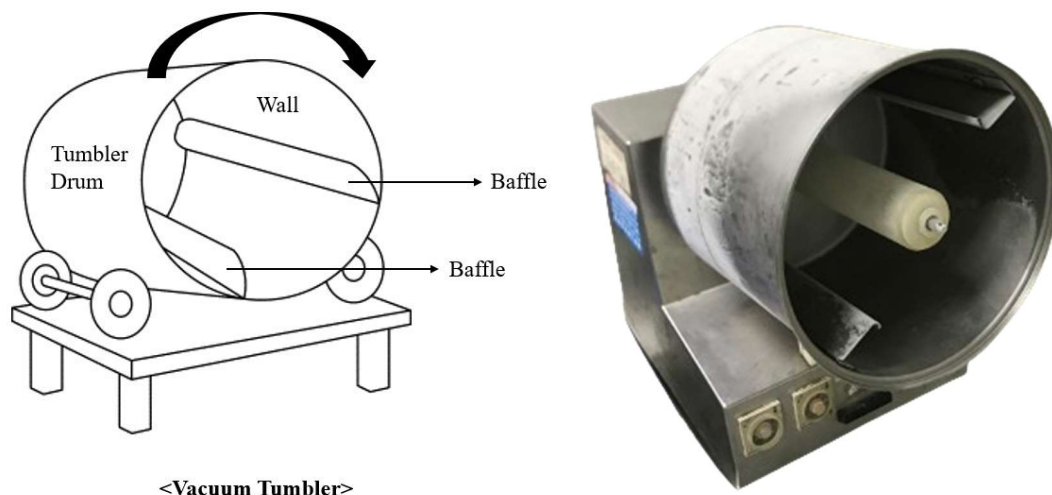


Figure 2-7. The visual of vacuum tumbler.
(Modified from Knipe, 2014).

2.3.3. Chemical tenderization

Chemical tenderization is a method of tenderizing meat by adding chemical compounds, agents, etc. through processes like injection, infusion, spraying, and marinating. According to a previous study, the early injection of calcium chloride into *Longissimus dorsi* from culled dairy cows can accelerate the tenderizing process throughout a week of aging (Bunmee et al., 2014), and it was indicated that salt and phosphate injection can assist beef eye of round muscles group to lead a 25 to 35%

reduction in shear power (Pietrasik et al., 2009). Despite these advantages, there have been numerous concerns raised about the undesirable flavor that may result from chemical injection treatments (Bunmee et al., 2014; Pietrasik et al., 2009). Additionally, regulations in some countries restrict using adding of certain chemical compounds on fresh meat (Long et al., 2011).

2.3.4. Plant protease in tenderization

Protease is an enzyme that breaks the peptide bond of a protein into smaller peptides. These proteolytic enzymes are often applied in meat processing to break down proteins in muscle tissues and hydrolyze collagen and elastin, contributing to meat tenderization (Rawdkuen et al., 2013). Proteases have a long history of use and some have received official classified as Generally Recognized as Safe (GRAS) by the FDA, USA. Among the various types of proteases, plant proteases are preferred and gains much attention of professionals in food science field (Fernández-Lucas, 2017). This preference is attributed by considerations related to religious requirements such as halal and kosher, as well as concerns about the potential possibility of transmission of waterborne infectious diseases associated with animal-based proteases (Morellon-Sterling et al., 2020; Gagaoua et al., 2021). Papain, ficin, bromelain, and malt are plant proteases that have received approval as Generally Recognized as Safe (GRAS) for use in the meat industry by the US Department of Agriculture. Among these, papain and bromelain are frequently chosen for meat tenderization (Ha et al., 2012; Liu et al., 2018). The descriptions of these two major plant enzymes are shown below:

i) Papain (EC 3.4.22.2):

Papain is a plant protease derived from the latex of *Carica papaya*, and it protects papaya from insects in nature (Konno et al., 2004). Papain is a highly researched and commonly used protease in the global food industry (Fernández-Lucas et al., 2017). Commercial papain has an acute oral toxicity dose of 10 g/kg body weight in mice. Therefore, papain has granted GRAS status by FDA since 1997 (Bekhit et al., 2014). Papain is a protein enzyme with a molecular weight of 23.4 kDa and 212 amino acid residues. It can break down almost every kind of peptide bond with a wide range of pH levels from 3.0 to 12.0, and the most efficient pH level is between 6.5 and 7.5. The optimal temperature for papain activation is 65°C, which is considered higher than other traditional enzymes (Smith & Hong, 2011; Gagaoua et al., 2021). It is importantly noted that papain enzymes can stay active even after cooking, which can lead to changes in food texture (Homaei et al., 2010). Papain is an enzyme that can function in a wide range of pH levels with relatively strong heat tolerance, making it suitable for diverse industrial applications. Papain is a powerful tenderizer that requires only a small amount (less than 0.01 AU/100 g) to have a significant effect. However, the broad specificity and random tenderization of proteins by papain can produce a mushy texture for food. When it comes to tenderizing the meat surface, it can develop the characteristic "mushiness" (Islam & Molinar-Toribio, 2013).

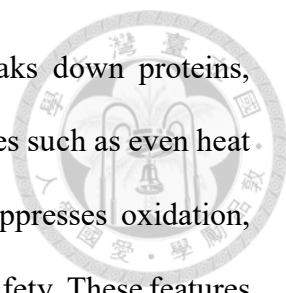
ii) Bromelain (EC 3.4.22.32):

Bromelain is a protease found in pineapples, specifically in both the fruit and stem. Bromelain consists of 285 amino acids residue, has a molecular weight of 33 kDa, an optimal pH range of 6.0 to 7.0, and an optimal temperature of around 50°C (Babu et al., 2008; Gagaoua et al., 2021). Bromelain, like papain, has an excessive protease action that may lead to over-tenderization. However, compared to papain, which breaks down

myosin and actin at equivalent rate, bromelain mainly breaks down myosin (Gagaoua, 2021). Moreover, bromelain is more effective in breaking down connective tissues, especially the heat-denatured collagen formed during cooking (Gagaoua, 2021). Bromelain can offer benefit of ensuring the microbiological quality and purity in industries with controlled environment (Arshad et al., 2016). Besides of its role in meat tenderization, bromelain is also used in various field such as pharmaceuticals, cosmetics, defecation of beer, and baking cookies (Mohan et al., 2016).

2.3.5. Thermal tenderization and Sous vide

Thermal tenderization is a process that makes meat tender by inducing protein denaturation through heating. Heating causes structural changes in meat protein (Berhe et al., 2014). The terrestrial structure of proteins is known to denature at temperatures of 50-60°C or higher, while the secondary structure, a component of the tertiary structure, denatures at temperatures of 60-90°C when exposed for at least one h (Farjami et al., 2021). However, an excessive heating at a temperature exceeding 100°C for an extended period can lead to over-denaturation of proteins. This can result in aggregation, where the binding force between proteins increases, potentially interfering with protein degradation by enzymes (Santé-Lhoutellier et al., 2008). Hence, it is crucial to pay a careful attention to cooking time and temperature when engaging in heating of meat (Berhe et al., 2014). In that context, Sous vide was specifically designed to compensate for potential disadvantages from heating. The term “sous-vide” originates from the French phrase “under vacuum,” denoting a cooking method that involves vacuum-packing food and cooking it under constant heat in water (Schellekens, 1996; Karki et al., 2022). Sous vide offers unique advantages compared to traditional heat treatment methods. Maintaining a constant heating temperature ensures uniform and desirable



tenderness. Cooking at ideal sous-vide temperatures properly breaks down proteins, resulting in an excellent texture. Vacuum packing provides advantages such as even heat transfer, longer shelf life, and low oxygen concentration, which suppresses oxidation, inhibits the growth of aerobic microorganisms, thus enhancing food safety. These features make sous vide the most preferred thermal tenderization for producing high-quality ready-to-eat meat products (Baldwin, 2012; Silva et al., 2016). To effectively utilize sous vide cooking, specific equipment is essential. The three basic elements of a sous vide machine include the following devices (**Figure 2-6**) (BC Centre for Disease Control, 2017):

- i. PID controller: A device or controller operates sous vide machine, ensuring proper additional device can be added with a temperature sensor and timer for accurate control of cooking conditions.
- ii. water circulator: A device circulates water within the equipment, promoting temperature uniformity.
- iii. heater: A device designed to heat water. Linking the heater with the temperature sensor allows for the maintenance of a constant temperature throughout the cooking.



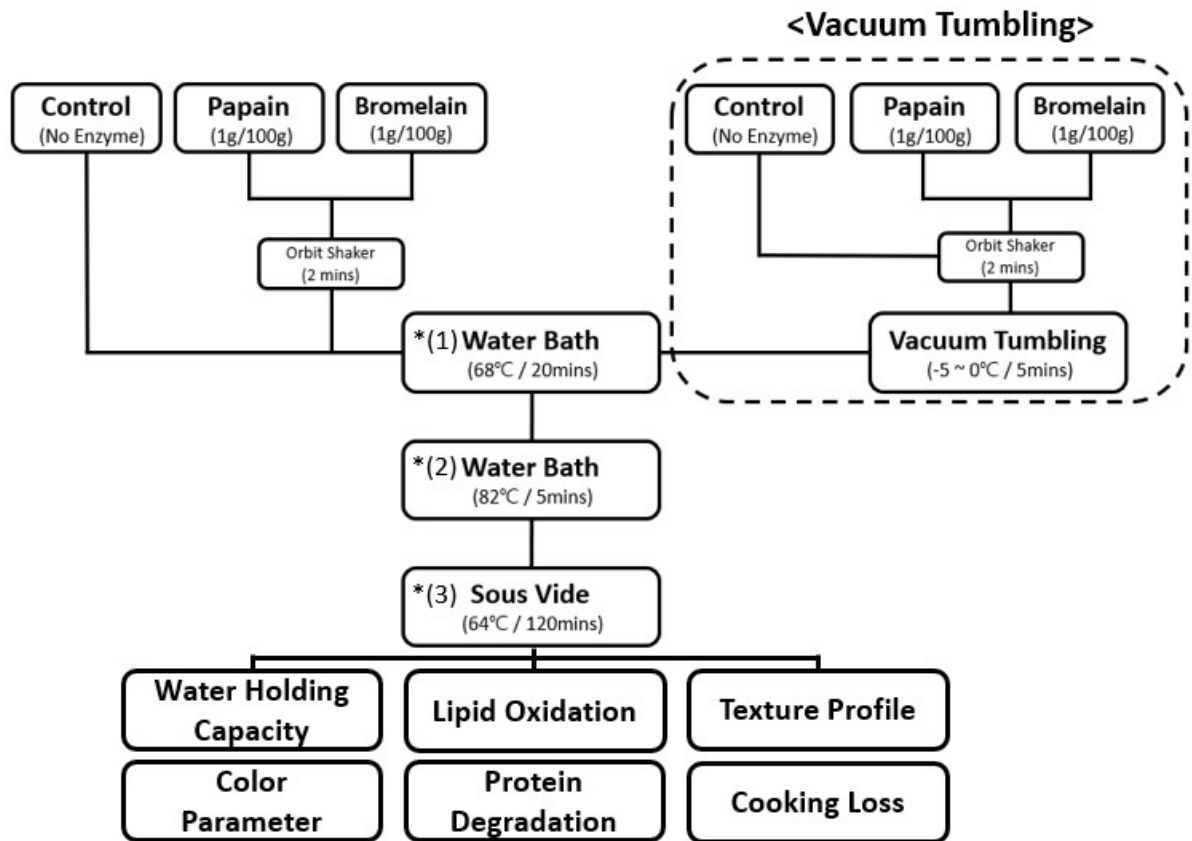
Figure 2-8. Details of sous vide machine (SVC107L, LAICA, Italy).

Additionally, there is a specific standard for vacuum pouches for sous vide cooking. The pouches must be thick enough to prevent leakage and impermeable to oxygen. To avoid oxygen transmission, the pouch should be a minimum of two millimeters thick. It is important to choose the appropriate pouch for the cooking method since different types of plastic have varying levels of oxygen transmissibility (BC Centre for Disease Control, 2017).

III. Materials and methods



3.1. The flow chart of the experimental design



* Temperature setting: based on Botinestean et al. (2018).

Figure 3-1. The flow chart of the experimental design.



3.2. Sample preparation

3.2.1. Raw material

Beef (Shoulder clod, Choice level, USA) was purchased from local retail stores (Costco, Taipei, Taiwan) for use in the experiment. After refrigerating the meat for one day, all the surface fat and heavy connective tissue were trimmed. For enzymes for the experiment, Papain (EC 3.4.22.2, Enzybel International, Belgium) and Bromelain (EC 3.4.22.32, Por-sheng, Taiwan) were selected.

3.2.2. The sample preparation for non-tumbling group

Beef clod was cut into nine steaks with a uniform thickness of 2.54 cm and a weight of 100 g. These steaks were then divided into three groups for each of the three replicated experiments. The treatment consisted of three groups (A of **Figure 2-2**):

- i. Control (C) without any enzyme treatment
- ii. Papain treatment (P) with 0.1% papain concentration
- iii. Bromelain treatment (B) with 0.1% bromelain concentration

The steaks were marinated in resealable plastic zip-lock bags with 0.1% concentrations of plant proteases. To ensure even distribution of the enzyme during marination, the mixture underwent uniform mixing for 3 minutes using an orbital shaker (Model OSR201-01, Genepure, Taiwan). After the enzymatic treatment, the steaks were cooked in a sous-vide thermal circulator (Model SVC107L, LAICA, Italy) at 68°C for 20 mins for enzyme activation. Enzyme inactivation is followed by heating in a circulation water tank (Model BT-150D, YIHDER, Taiwan) at 82°C for 5 mins. Then, all samples were vacuum-packaged and cooked using the sous-vide thermal circulator (Model SVC107L, LAICA, Italy) at 64°C for 120 mins (Botinestean et al., 2018). The control

group (C) was treated the same way as the other test group, except for the addition of enzymes. All steaks were then stored at -16°C overnight.



3.2.3. The sample preparation for tumbling group

Tumbling group underwent the same treatment as the non-tumbling group, except for the tumbling process. Tumbling was carried out immediately after marination, using a vacuum tumbler (Model MGH-20, DORIT, Switzerland) at 25 revolutions per minute. The groups that underwent tumbling were as follows (B of **Figure 3-2**):

- i. Control group with 5 minutes of tumbling (CVT)
- ii. Papain treatment group (0.1/100g) with 5 minutes of tumbling (PVT)
- iii. Bromelain treatment group (0.1/100g) with 5 minutes of tumbling (BVT)

After tumbling, all samples were vacuum packed, the steaks were cooked in a sous-vide thermal circulator (Model SVC107L, LAICA, Italy) at 68°C for 20 mins for enzyme activation. Enzyme inactivation is followed by heating in a circulation water tank (Model BT-150D, YIHDER, Taiwan) at 82°C for 5 mins. Then, all samples were vacuum-packaged and cooked using the sous-vide thermal circulator (Model SVC107L, LAICA, Italy) at 64°C for 120 mins (Botinestean et al., 2018). The control group (C) was treated the same way as the other test group, except for the addition of enzymes. All the steaks were then stored at -16°C overnight.

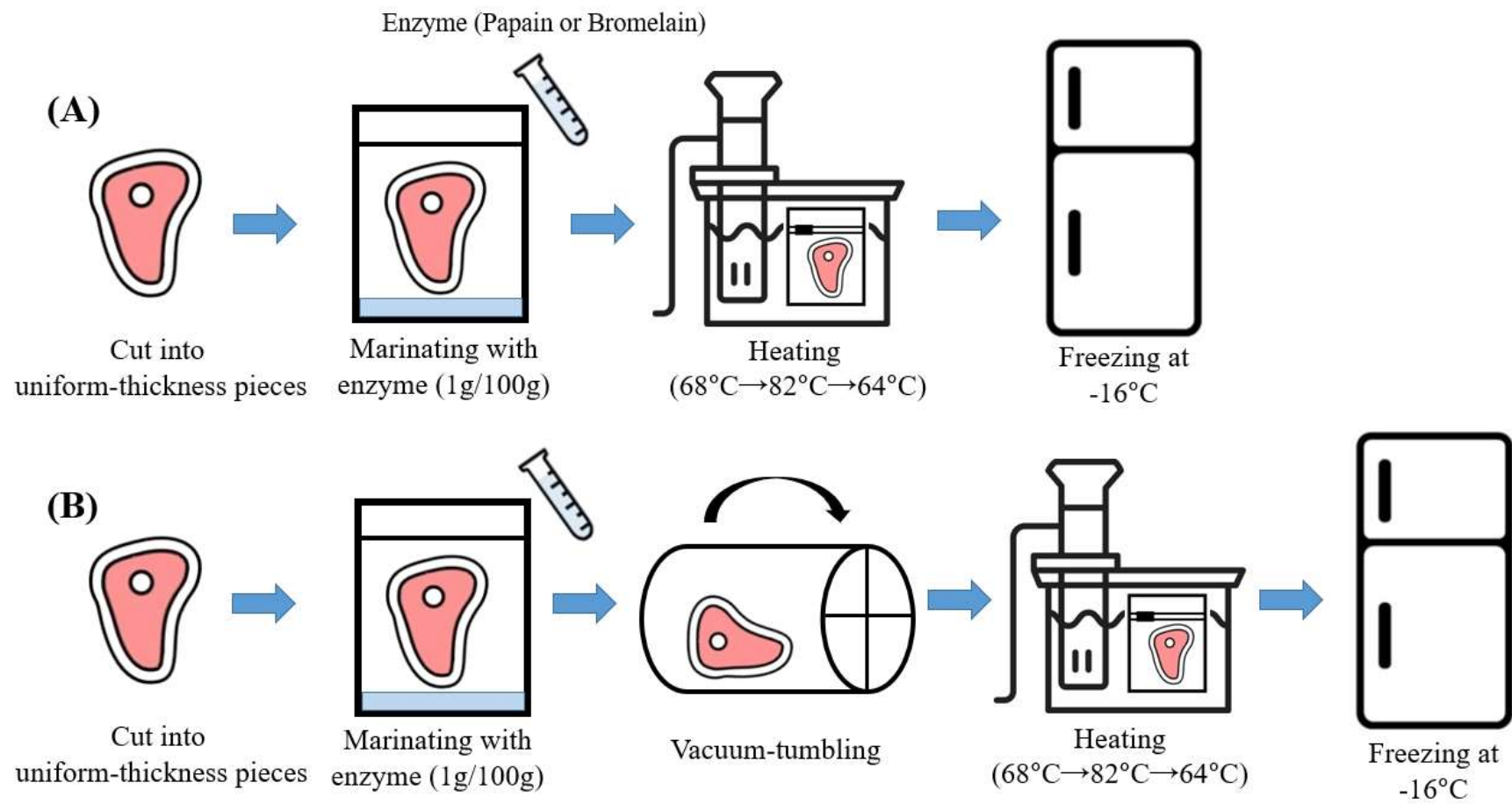


Figure 3-2. The process of sample preparation.

3.3. Physicochemical properties measurement



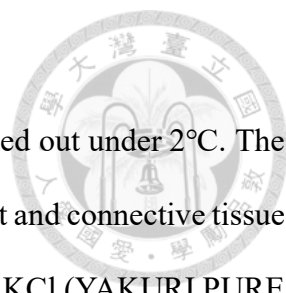
3.3.1. Texture profile analysis of tenderized beef clod

Texture profile analysis (TPA) was conducted on the samples that undergone all treatments after cooling under running water. Samples were cut into cubes of 1 cm³ (1 × 1 × 1 cm). These cubes were vertically compressed by using cylinder-type probe P/50 (50 mm diameter cylinder aluminum, Stable Micro System Ltd., Godalming, UK) and then data were calculated by a texture analyzer machine (TA. XTplus, Stable Micro System Ltd., Godalming, UK). The analysis was conducted more than three times for each group of samples under 5 mm/ sec of speed with 50 % of strain (**Table 3-1**).

Table 3-1. Texture analyzer setting for texture analyzing sequence in the present study.

Caption	Value	Units
Pre-Test Speed	1.00	mm/sec
Test Speed	5.00	mm/sec
Post-Test Speed	5.00	mm/sec
Target Mode	Strain	
Strain	50	%
Time	5	sec
Trigger Force	5.0	g

3.3.2. Myofibrils fragmentation of tenderized beef clod

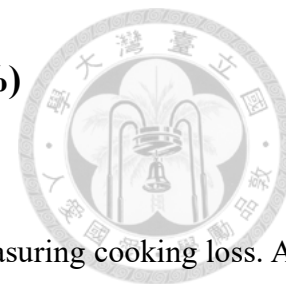


The entire MFI (Myofibril Fragmentation Index) process was carried out under 2°C. The process involved taking 4 g of samples without any visible amounts of fat and connective tissue, which were then placed in 40 mL of MFI buffer, a mixture of 7.455 g of KCl (YAKURI PURE CHEMICALS CO., LTD., Japan), 0.37224 g of EDTA (Mallinckrodt, Ireland), 2.8392 g of Na₂HPO₄ (Wako Pure Chemical Industries, Ltd.), 3.1202 g of NaH₂PO₄ (Wako Pure Chemical Industries, Ltd.) and 1 L of double-distilled water (ddH₂O). The mixture was homogenized using a homogenizer (Mini-BeadBeater-16, BIOSPEC, USA) for 30 seconds. The pellet of myofibrils was collected and centrifugated (Centrifuge 3700, Kubota Corp., Japan) at 1,000×g for 15 minutes. The pellet was resuspended with 40 mL of MFI buffer and centrifuged at 1,000×g for 15 minutes. The final pellet was collected again, resuspended with 5 mL of MFI buffer, and vortexed. The final homogenate was filtered using a glass stirring rod through a plastic filter with an 80 µm pore size. The protein concentration of the homogenate was measured using ELISA (Hybrid Reader, Synergy H1, Biotek Inc., USA). The absorbance of the suspension (0.5 mg/mL) was measured at 540 nm with triplication.

3.3.3. Cooking loss of tenderized beef clod

All beef clod samples were cut to a thickness of 2.5 cm and weighed exactly one hundred grams. The untreated meat was weighed accurately as W_1 before any treatment. After that, the sample that underwent pre-sous vide treatment and sous vide cooking was weighed after cooling under running water, and this was recorded as W_2 . Cooking loss was calculated by expressing it as a percentage of W_1 and the difference before and after cooking. The formula used to calculate the cooking loss is as follows:

$$\text{Cooking Loss (\%)} = \frac{W_1 - W_2}{W_1} \times 100 (\%)$$



3.3.4. Expressible moisture (EM) of tenderized beef clod

Samples are cut into smaller pieces with 0.2 g of weight after measuring cooking loss. A piece of the sample was placed in a centrifugation tube (1.5 ML, Eppendorf, Germany) with a resized laboratory filter paper (90mm, ADVANTEC, Taiwan) for absorbing moisture and centrifuged at 1100 rpm for 1 hour. After that, the sample was completely removed and the percentage of expressible moisture (%) was calculated using the following equation.:

$$\text{EM (\%)} = \frac{\text{Final weight of filter paper (g)} - \text{Initial weight of filter paper (g)}}{\text{Sample weight (g)}}$$

3.3.5. Lipid oxidation of tenderized beef clod

The lipid oxidation of beef clod was measured by thiobarbituric acid reactive substances (TBARS). All samples and chemicals were kept on ice to prevent oxidation. First, 1g of the sample was mixed with 9 mL PBS in a tube and homogenized by using a homogenizer (Mini-BeadBeater-16, BIOSPEC, USA). The mixture was centrifuged (Centrifuge 3700, Kubota Corp., Japan) at 3000rpm at 4°C for 15 minutes, and the supernatant was extracted from the tube. 60μL of the supernatant was mixed with 90μL of TBA solution and 510μL of TCA-HCL solution. The mixture was incubated in a circulation water tank (Model BT-150D, YIHDER, Taiwan) at 95°C for 30 minutes, then cooled on ice for 5 mins. Finally, the mixture was centrifuged (Centrifuge 3700, Kubota Corp., Japan) at 10,000rpm at 4°C for 3 minutes. For the experiment, 200mL of the mixture was measured using ELISA (Hybrid Reader, Synergy H1, Biotek Inc., USA). The absorbance of the mixture was measured at 535 nm TBARS value (mg MDA eq./kg of sample) from the result. The TBARS value was calculated by the following equation:



$$\text{g MDA eq./kg beef clod} = \text{Sample } A_{535} \times 5.082 \times 10^{-2}$$

3.4. Color parameter of tenderized beef clod

Color evaluation was conducted by a color checker (Model NR-11, Nippon Denshoku, Japan) that uses the CIE $L^*a^*b^*$ system (**Figure 3-3**). Each L^* , a^* , and b^* value indicates the lightness, redness, and yellowness of the sample. Before the evaluation, the color checker was calibrated using a calibration procedure. For each sample, three replicated data were taken from the surface, and the average values of the lightness (L^*), redness (a^*), and yellowness (b^*) parameters were recorded. The total color difference was calculated in comparison with uncooked raw meat. L^* , a^* , b^* indicates the color-parameter values of raw meat, and L , a , and b indicates the color-parameter values of the sample. The equation of total color difference (ΔE) is as follows:

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2}$$

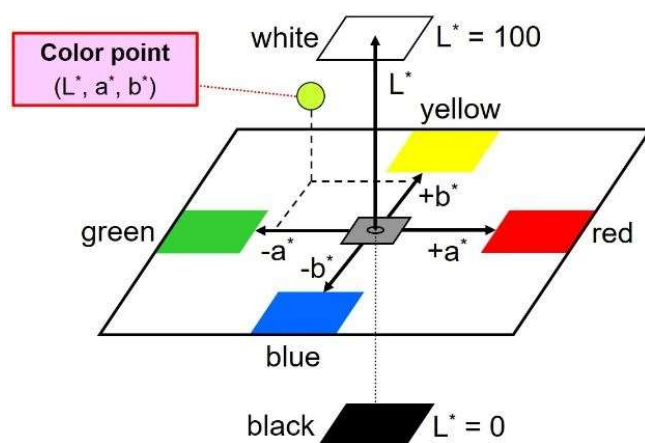


Figure 3-3. The three-dimension of CIE $L^*a^*b^*$ color space.

(Source: <https://www.ulprospector.com/knowledge/10780/pc-the-cielab-lab-system-the-method-to-quantify-colors-of-coatings/>)



3.5. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

3.5.1. Sample preparation for SDS-PAGE

1 g beef clod sample was mixed with 3 mL of phosphate-buffered saline (PBS, pH 7.0) and homogenized using a homogenizer (Mini-BeadBeater-16, BIOSPEC, USA) for 30 sec. The mixture was centrifuged at 4000 rpm at 4°C for 10 minutes, and the supernatants were collected. For the experiment, 14 µl of the supernatant, 5 µl of 4X Laemmli sample buffer, and 1 µl of 1 mol DTT (DL-Dithiothreitol, Sigma-Aldrich Co., LLC, USA) were mixed in a 1.5 mL centrifuge tube. The mixture was heated in a circulator water bath at 95°C for 10 minutes, then cooled on ice for 10 minutes. The samples were remixed by using a vortex mixer (Mini Centrifuge Force Mini, Select Bioproducts, USA), centrifuged at 1000 rpm for 10 seconds after the sample sufficiently cooled, and utilized it in the experiment.

3.5.2. Electrophoresis

Double-distilled water (ddH₂O), 1.5 mol of Tris-HCl (pH 8.8), 30% acrylamide, and 10% SDS were mixed together to prepare a running gel. Then, TEMED and 10% ammonium persulfate (APS) were added just before the gel preparation to prevent the gel turn hard. The running gel was prepared by placing it in a frame and adding ethanol (EtOH) to prevent bubble forming. The gel was left for about 10 minutes until it hardened, then the ethanol on the top was removed. To prepare the stacking gel, ddH₂O, 1.0 mol of Tris-HCl (pH 6.8), 30% acrylamide, 10% SDS, TEMED, and 10% ammonium persulfate (APS) were mixed together. The stacking gel was casted into the top of the running gel, and a well comb was placed and hardened to complete the process. After filling the gel with running buffer (Tris 3.03g, Glycine 14.4g, SDS 1g in 1L DDW), 10 µl of sample and 2 ul of protein marker (Precision Plus Protein

All Blue Prestained Protein Standards, Bio-Rad, USA) were dispensed into each well from the edge. Electrophoresis was conducted using a Power supplier (MP-250V, Major Science, USA) at 80V for 40 minutes. The voltage was then switched to 120V for 90 minutes until the marker reached the bottom of the gel. After electrophoresis was complete, the gel was washed for 5 minutes using DW, and the washing process was repeated three times. Then the gel was stained with 0.06% (w/v) Coomassie Brilliant Blue G-250 (Sigma-Aldrich Co., USA) for 1 h and then destained for 3 h with methanol.

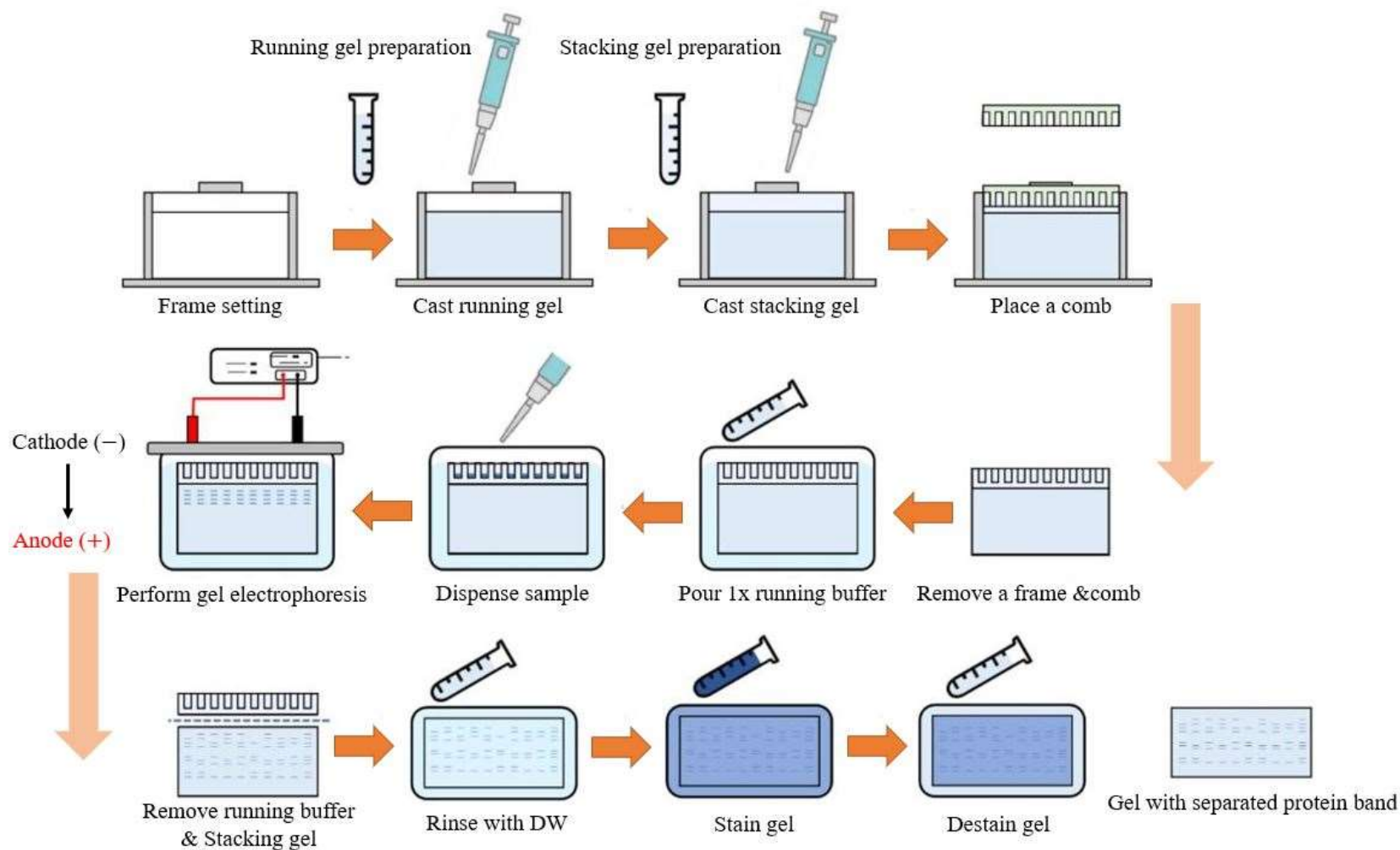
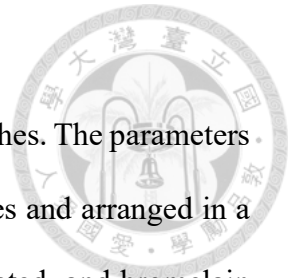


Figure 3-4. The procedure of SDS-PAGE analysis.

3.6. Statistical analysis

All analyses were conducted for three independently treated batches. The parameters for each tested batch per product were obtained with at three analyses and arranged in a 2 (non-tumbling and tumbling) $\times$ 3 (non-enzyme treated, papain treated, and bromelain treated) factorial design to obtain the results concerning the physicochemical properties of beef clod cooked by sous vide. The interaction effect of tumbling effect and enzyme treatment, as well as two main effects (tumbling or enzyme), were analyzed. Differences were considered to be significance at a p value of 0.05. When a significant difference in the interaction effect was found, Least Significant Difference (LSD) test at $p < 0.05$ was used to assess differences between combination treatments. All statistical analyses of data were performed using SAS 9.2 software (SAS Institute Inc., Cary, NC).



IV. Result and discussion



4.1. Physicochemical properties measurement

4.1.1. Texture profile of tenderized beef clod

Tenderness is an important element in East Asian cuisine, often determining the commercial value of meat in the market (Chambers & Bower, 1993; Nam et al., 2010). **Table 4-1** shows the results of the texture profile analysis (TPA) conducted on tenderized beef clod. The study found no significant differences between the non-tumbling and tumbling groups ($p>0.05$), regardless of the parameters. Previous research by Tuell et al. (2015) showed that tumbling without any pre-treatment can lead to improved tenderness (60, 90 min at 8.5 rpm), while Gao et al. (2015) investigated tumbling with pre-marinated pork samples (8 h, 11 revolutions per minute) and found decrease on various Texture Profile Analysis (TPA) parameters. However, the present study showed no significant effect of tumbling, which is similar to the findings of Morrow et al. (2019). This lack of impact was attributed to the lower amount of cumulative revolutions applied during the tumbling process, which was only 5 minutes, 25 revolutions per minute in the present study. As a result, statistical analysis was conducted by combining the non-tumbling and tumbling groups for each enzyme group, with a sample size of ($n=3$). On the other hand, there was a significant difference between the enzyme treated group (Papain, Bromelain) group and non-enzyme treated group in Hardness, Cohesiveness, Gumminess, and Chewiness. Among two enzymes, Bromelain group, Especially, had the lowest value in hardness, gumminess, and chewiness. These findings are consistent with the studies carried out by Zhang et al. (2019), and Xu et al. (2020) which had a result of low hardness and related TPA parameter value due to using plant protease as a tenderizer. The reason of hardness decrease can be explained by the action of papain and bromelain, which cause

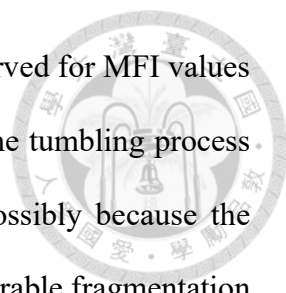
fragmentation of myofibrillar proteins and disruption of connective tissue (Xu et al., 2020). Gerelt et al. (2000) also found that the tenderness of muscle was improved by the proteolytic removal of Z-lines in plant protease-treated samples. Thus, the results clearly demonstrate that two plant proteases (papain, bromelain) significantly improve beef clod tenderness and can be effectively utilized to improve tenderness.

The bromalin-treated group showed lower hardness than the papain-treated group, which is different from the result of Botinestean et al. (2017). The difference in results might be because of the different chemical composition of beef cut (Oh et al., 2016) and it has been found that the hardness of meat is related with its connective tissues. Bromelain has specificity on connective tissue, so it is more effective on breaking down connective tissues compared to papain (Gagaoua et al., 2021; Pematilleke et al., 2022).

However, for springiness and cohesiveness, the papain group had the lowest value. This can be explained by papain action which cause hydrolysis of myofibrils and leading to moisture loss in the internal structure of muscle (Zhang et al., 2019). According to a study by Gagaoua et al. (2021), papain has a greater effect on myofibrillar proteins than bromelain. This is important because the movement of water in meat is affected by changes in myofibril spacing, as noted by Pearce et al. (2011). As a result, papain may have influenced the springiness and cohesiveness of the meat.

4.1.2. Myofibrillar fragmentation index (MFI) of tenderized beef clod

Myofibrillar Fragmentation Index (MFI) indicates the integrity of myofibrils (Cheng et al., 2021). MFI of the present study is illustrated **Figure 4-2**. A significant increase in MFI values was observed between C and CVT, this increase is caused by the physical disruption caused by tumbling, leading to the fragmentation of myofibrils with unmeasurable proteolytic degradation, which is aligning with findings in the study



conducted by Tuell et al. (2021). In contrast, no significant was observed for MFI values of P and PVT, as well as B and BVT ($p>0.05$). This suggests that the tumbling process did not induce additional tenderizing effect in the MFI values, possibly because the enzyme action and sous vide cooking had already progressed considerable fragmentation of myofibrils. The enzyme-treated group shows a higher MFI value compared to the non-enzyme group. However, there was no significant difference observed between the papain and bromelain treatments ($p>0.05$). Higher MFI values suggest that the myofibrillar structure has undergone more extensive proteolysis, which is strongly related with meat tenderness (Aroeira et al., 2020). This is consistent with the reduction in hardness, stickiness, and chewiness seen in **Table 4-1** and the MFI result of Xu et al. (2020).

4.2. Meat quality of tenderized beef clod

4.2.1. Cooking loss

Cooking loss value of the present study is illustrated in (A) of **Figure 4-1**. No significant difference was observed between the non-tumbling group and the tumbling group ($p>0.05$). Although Dzudie et al. (1999) found a decrease in cooking loss with increasing tumbling time (2, 4, or 6 hours at 28 rpm) in the experiment applying tumbling to goat ham, the present experiment found no significant difference between non-tumbling group and the tumbling group due to insufficient tumbling time and the number of revolutions (5 minutes, 25 revolutions per minute). Therefore, the statistical analysis proceeded with sample size of ($n=3$), distinguishing only between groups with non-enzyme-treated group and two different enzymes. Cooking loss refers to the amount of liquid and soluble matter that is lost from the meat during the cooking process (Aaslyng et al., 2003). The increase of cooking loss in enzyme-treated group can be attributed to

the disaggregation and degradation of myofibrillar proteins, as well as the movement of water molecules from these proteins into the extracellular space (Xu et al., 2020). This finding has been supported by previous studies conducted by Botinestean et al. (2015) and Xu et al. (2020), which also reported increases in cooking loss. The cooking loss measured in this study was comparable to those reported by Botinestean et al. (2021), ranging from 29-36.71% (pre-enzyme treatment + sous vide 120 mins), and a similar range of 34.47-37.37% (pre-enzyme-treatment + sous vide 120 - 270 min).

4.2.2. Expressible moisture (EM)

Expressible moisture (EM) value of the present study is illustrated in **(B)** of **Figure 4-1** and it shows that B had the highest EM. The reason perhaps is B had the highest MFI (**Figure 4-2**) and the higher the degree of fragmentation of structural proteins led to lower the water holding capacity (Istrati et al., 2012).

An increase in EM was observed between C and CVT and between P and PVT. However, this result differs from the study conducted by Pietrasik et al. (2021), which showed that incorporating tumbling (2 h) into moisture-enhanced beef roasts resulted in a decrease in EM. Expressible moisture (EM) is a method measuring the moisture retained in the cooked product. The reason for this difference could be attributed to the physical impact of tumbling on the sample, as it may have had a greater effect on EM than the effect of improvement in water holding capacity (WHC) because of the short duration of tumbling (5 mins). For comparison of non-enzyme-treated group and the enzyme-treated group, the enzyme-treated group (P, PVT, B, and BVT) had higher Expressible Moisture (EM) values. It has been observed that cooking loss (**A** of **Figure 4-1**) and EM are inversely related (Ji et al., 2019). However, the use of papain and bromelain for tenderizing meat can have a negative impact on WHC by reducing the amount of water bound with changes

in myofibrillar protein structure.



4.2.3. Lipid oxidation of tenderized beef clod

TBARS value of the present study is presented on **Figure 4-4**. In the present study, no significant TBARS values between non-enzyme treated group and enzyme-treated group was founded ($p>0.05$). Instead, triplication of TBARS values were classified into Non-tumbling group and tumbling group, and statistical analysis was performed with sample size ($n=27$). The tumbling-treated group had higher thiobarbituric acid reactive substance (TBARS) values than the non-tumbling-treated group ($p<0.05$). The thiobarbituric acid reactive substances (TBARS) assay can be used to measure the extend of lipid oxidation in meat samples. The higher the level of oxidized lipids, the higher is the TBARS value. Bishnoi et al. (2017) reported the tumbling process may promoted lipid oxidation by damaging the cell membrane where the phospholipids are located. This can create a pathway for free radicals or catalysts to oxidize the phospholipids, thus making it easier for oxygen to penetrate the meat structure when tumbling is done without vacuum. It was found in the previous MFI and EM (**Figure 4-2; B of Figure 4-1**) results that vacuum tumbling could not produce desirable effect due to the short duration of the process. Moreover, there were some expected physical impacts on the content inside the drum as observed through the results of MFI and EM (C, CVT). Thus, there is possibility that the unmeasurable physical effects within the drum might have affected the TBARS value.

4.3. Color parameter of tenderized beef clod

The results of the present experiment revealed no significant difference between the non-tumbling group and the tumbling group ($p>0.05$). This was likely due to insufficient

tumbling time and the number of revolutions. Therefore, the groups were divided into enzyme-treated and non-enzyme treated, with a sample size of (n=3) for each group. The result of the experiment showed on **Table 4-2** that the enzyme-treated group had higher L* values compared to the non-enzyme group and bromelain group had the highest L* value. However, there was no significant difference for a* and b* values among all groups.

The degradation of myoglobin may result in the reduction of the absorption of a dark color (Razali et al., 2023). An increase in L* value was also observed in the study of Nadzirah et al. (2016). **Figure 4-2** depicts beef clod after sous vide cooking, showing that even a single piece of meat has a different color on different spot. The total color difference (ΔE) analysis indicated that the papain treatment group had the most similar color to the raw meat. Although the E value was lower than that of the other groups, the value is still $\Delta E > 5$ (Tkacz et al., 2020). People could notice that the papain-treated meat was different from raw meat.

4.4. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

analysis of different tenderizing method

The findings from the protein pattern analysis of SDS-PAGE are displayed in **Figure 4-5** After the enzyme treatment of P, PVT, B, and BVT, degradation of MHC around 250 kDa and actin protein around 45 kDa was observed. All treatment groups showed a similar tendency of proteolysis. It appears that the hydrolysis of muscle myofibrillar proteins influenced these results. These findings are consistent with the study of Xu et al. (2020). Moreover, the decrease in hardness (**Table 4-1**) and increase in MFI value (**Figure 4-2**) with the hydrolysis of muscle myofibrillar proteins may support the SDS-PAGE results. However, tumbling did not produce any significant changes in the experimental results.

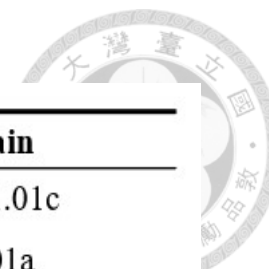


Table 4-1. Effect of enzymatic treatments on texture profile analysis (TPA).

Treatment	Control	Papain	Bromelain
Hardness (g)	1827.13 $\pm$ 54.80a	1234.24 $\pm$ 62.12b	856.50 $\pm$ 61.01c
Springiness	0.55 $\pm$ 0.01ab	0.52 $\pm$ 0.02b	0.59 $\pm$ 0.01a
Cohesiveness	0.60 $\pm$ 0.02a	0.49 $\pm$ 0.01b	0.57 $\pm$ 0.01a
*Gumminess (g)	1242.82 $\pm$ 20.20b	692.34 $\pm$ 44.23b	468.75 $\pm$ 47.36c
*Chewiness (g)	721.63 $\pm$ 15.66a	371.51 $\pm$ 16.78b	303.18 $\pm$ 35.59b

* The data are given as mean $\pm$ SEM (n=3). a-c Means within columns that do not share a common letter are significantly different (p<0.05).

** Hardness x Cohesiveness= Gumminess; Hardness x Cohesiveness x Springiness= Chewiness.

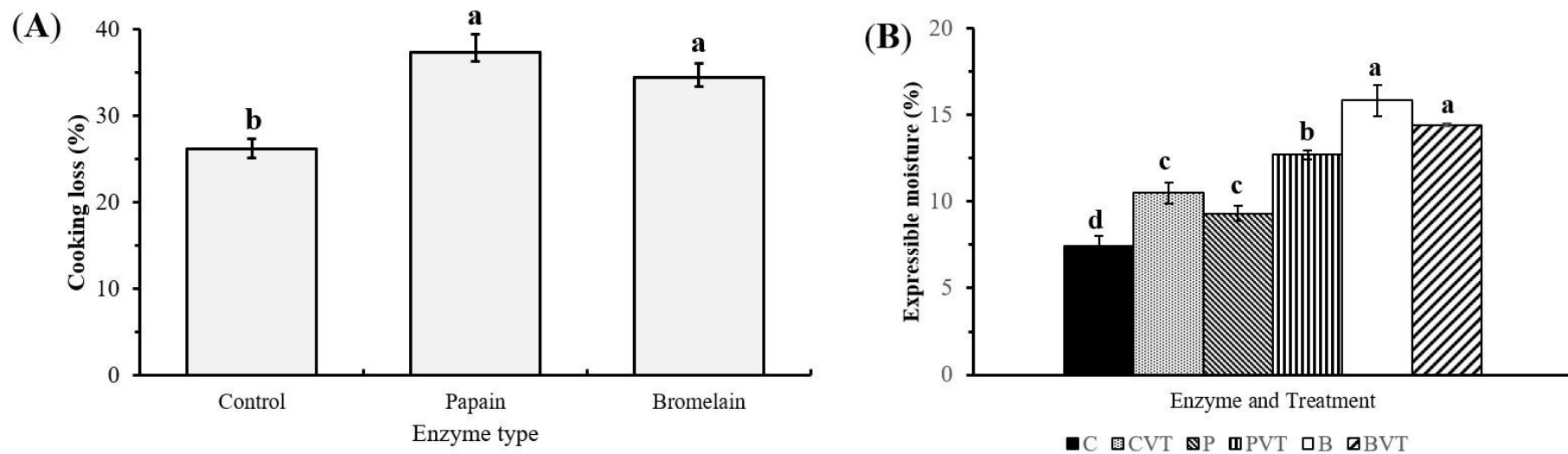


Figure 4-1. Cooking loss (A) and expressible moisture (B) of beef clods with different tenderizing treatments. C, Control Beef clod; CVT, Beef clod with 5mins of vacuum tumbling; P, Beef clod with papain tenderizing; PVT, Beef clod with papain and vacuum tumbling (papain solution+5mins of vacuum tumbling); B, Beef clod with bromelain tenderizing; BVT, Beef clod with bromelain and vacuum tumbling (bromelain solution+5mins of vacuum tumbling). The data are presented as mean $\pm$ SEM (n=3) (A) (n=6) (B). a-c Means with different letters are significantly different ($p < 0.05$).

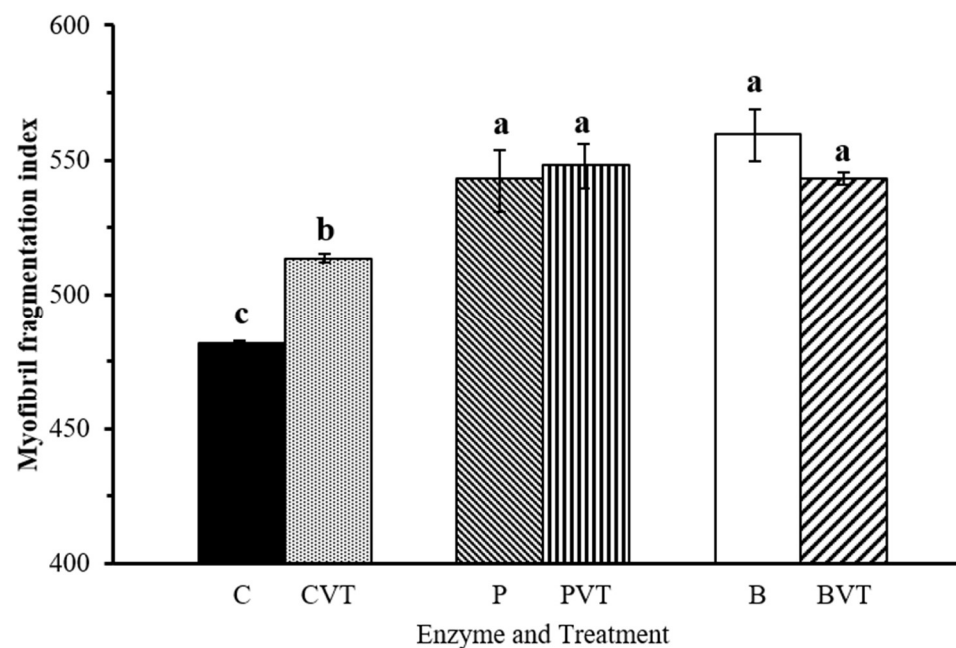


Figure 4-2. Myofibrillar fragmentation index (MFI) with different tenderizing treatments. C, Control Beef clod; CVT, Beef clod with 5mins of vacuum tumbling; P, Beef clod with papain tenderizing; PVT, Beef clod with papain and vacuum tumbling (papain solution+5mins of vacuum tumbling); B, Beef clod with bromelain tenderizing; BVT, Beef clod with bromelain and vacuum tumbling (bromelain solution+5mins of vacuum tumbling). The data are presented as mean $\pm$ SEM (n=3). a-c means with different letters are significantly different ($p < 0.05$).

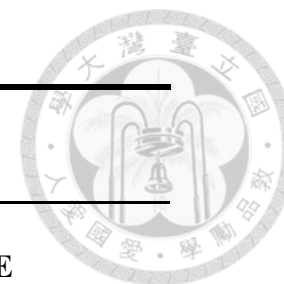


Table 4-2. Effect of enzymatic treatments on color parameters analyzed.

Treatment	Color parameters			
	Lightness (L*)	Redness (a*)	Yellowness (b*)	ΔE
Control	29.18 $\pm$ 0.30c	8.60 $\pm$ 0.30a	14.00 $\pm$ 0.42a	10.94 $\pm$ 0.29a
Papain	41.68 $\pm$ 0.43b	8.34 $\pm$ 0.67a	14.79 $\pm$ 0.55a	5.77 $\pm$ 0.50c
Bromelain	46.56 $\pm$ 0.81a	8.37 $\pm$ 0.44a	15.21 $\pm$ 0.63a	9.12 $\pm$ 0.71b

* The data are presented as mean $\pm$ SEM (n=3). a-c Means different letters within column are significantly different (p<0.05).

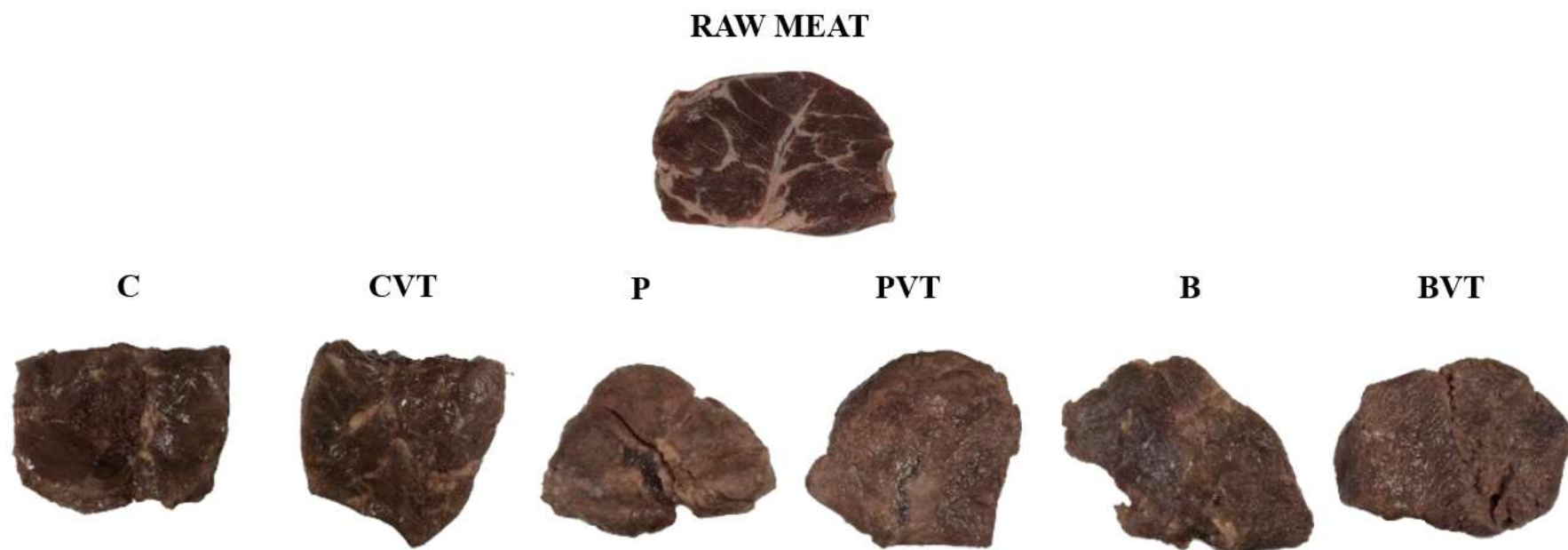


Figure 4-3. Visual appearance of tenderized beef clods.

* C: Control Beef clod; CVT: Beef clod with 5mins of vacuum tumbling; P: Beef clod with papain tenderizing; PVT: Beef clod with papain and vacuum tumbling (papain solution+5mins vacuum tumbling); B: Beef clod with bromelain tenderizing; BVT: Beef clod with bromelain and vacuum tumbling (bromelain solution+5mins vacuum tumbling).

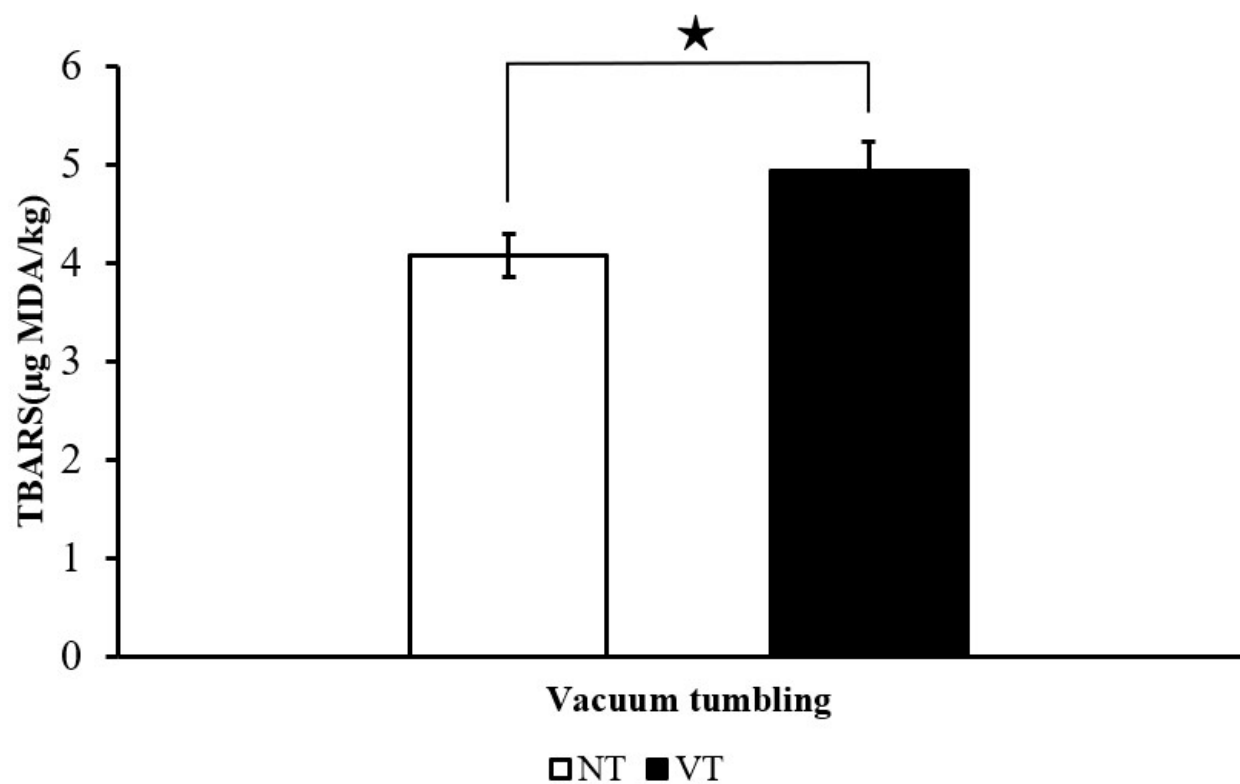


Figure 4-4. Effects of vacuum tumbling on lipid oxidation of treated samples.

* The data are given as mean $\pm$ SEM (n=27).

** ★ Mean values in each parameter were significantly different (p<0.05).

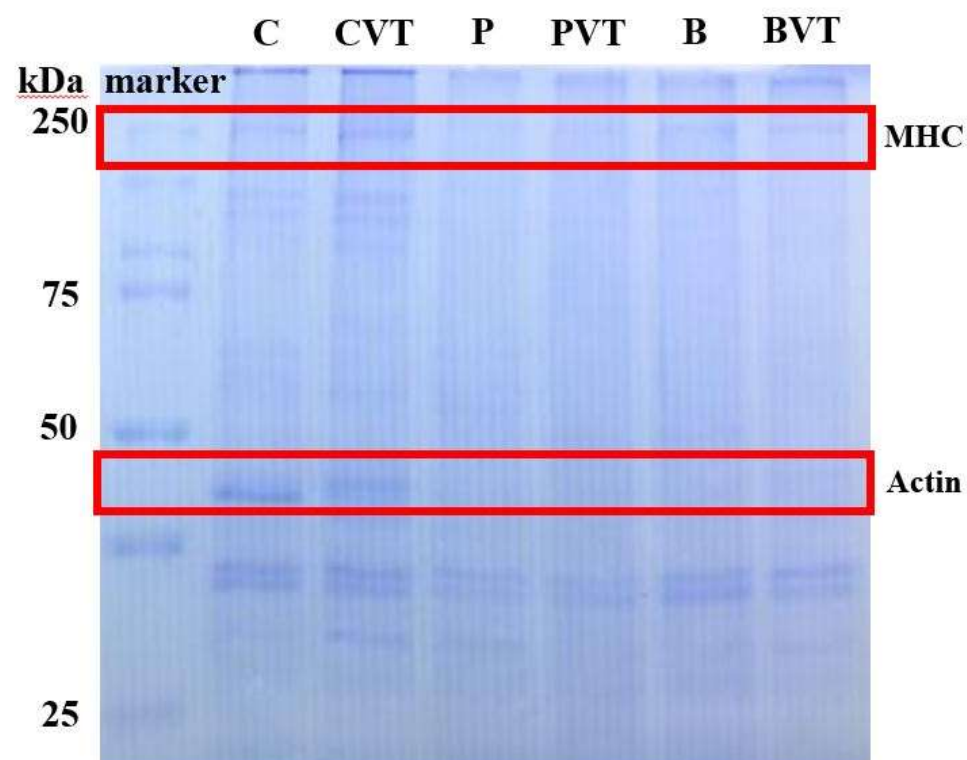
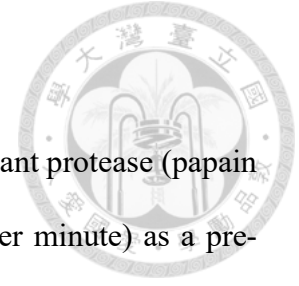


Figure 4-5. Protein patterns in the supernatant from various tenderizing treatments analyzed by SDS-PAGE.

* C: Control Beef clod; CVT: Beef clod with 5mins of vacuum tumbling; P: Beef clod with papain tenderizing; PVT: Beef clod with papain and vacuum tumbling (papain solution+5mins vacuum tumbling); B: Beef clod with bromelain tenderizing; BVT: Beef clod with bromelain and vacuum tumbling (bromelain solution+5mins vacuum tumbling)

V. Conclusion

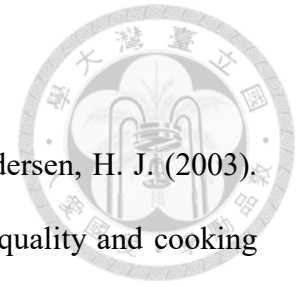


The purpose of this study was to examine the effects of using plant protease (papain and bromelain) and vacuum tumbling (5 minutes, 25 revolutions per minute) as a pre-sous vide treatment on beef clod for finding out the most effective method and enzyme. Previous studies have found that papain and bromelain can effectively decrease hardness and improve tenderness. In the present study, texture profile analysis (TPA) showed that bromelain resulted in the lowest hardness, gumminess, and chewiness, while papain resulted in the lowest springiness and cohesiveness. Both enzymes were found to be specific to certain proteins. In terms of tenderness (as measured by the Myobril fragment index or MFI), bromelain was found to have the highest value. This result was supported by the TPA results and the decomposition of MHC band in the SDS-PAGE analysis. cooking loss and expressive moisture tests were performed to evaluate the impact of the plant protease on water holding capacity. Both papain and bromelain resulted in high moisture loss compared to the control group. The expressive moisture test showed that the bromelain treatment group had the highest value, which suggests that this group may be more susceptible to losing moisture due to external impact caused by the fragmentation.

Furthermore, color parameter analysis revealed that the L^* value of the enzyme-treated group was significantly higher compared to the non-enzyme-treated group. This may be due to the decomposition of myoglobin. There was no noticeable difference between the enzyme-treated and non-enzyme ($p>0.05$) treated groups in the measurement of TBARS (which evaluates lipid oxidation). Instead, we compared the tumbling-treated and non-tumbling-treated groups and found that the tumbling-treated groups had higher TBARS values. This suggests that short periods of tumbling did not effectively prevent oxidation, and that physical impacts inside the drum may have disrupted the cell membrane and affected free radicals and catalysts that cause oxidation of phospholipids.

Overall, the short time (5 minutes) of vacuum tumbling did not result in the expected conventional vacuum tumbling effect, and instead led to a decrease in the quality of the meat. However, plant protease treatment was found to be an effective method for tenderizing beef clod, which is typically tough due to a lot of connective tissue. Bromelain was found to be particularly effective. The current study proposes that bromelain could serve as a promising tenderizer, particularly in the East Asian market where tenderness holds significant value. This finding is considered valuable, as it presents a potential method that could also be applied to the development of elderly-friendly food.

VI. Reference



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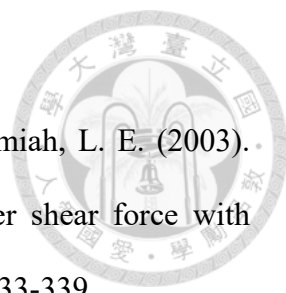
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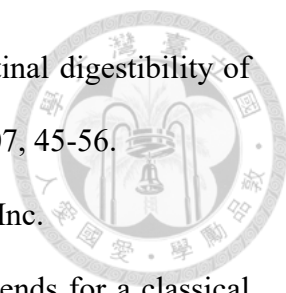
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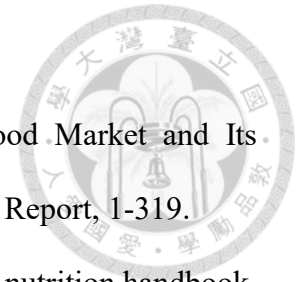
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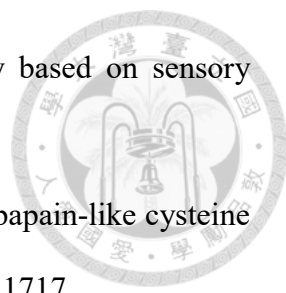
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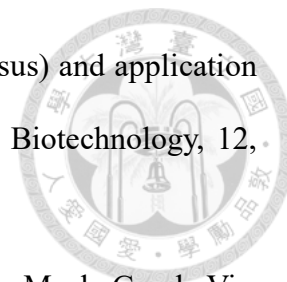
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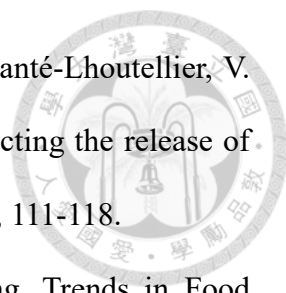
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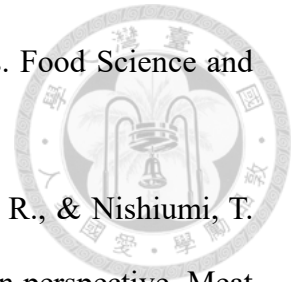
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VII. Appendix



Master's thesis

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