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Effect of Vacuum Impregnation on the Quality of Beef Cubes Marinated with Various Natural Meat Tenderizing Agents

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ABSTRACT

This study investigates vacuum impregnation technology, a non-thermal approach to treat local beef cubes (2 cm × 2 cm × 2 cm) with three distinct marinades: distilled water (CONTROL), 10% (v/v) pineapple juice (PINE), and 10% (v/v) papaya juice (PAPAYA), both containing plant proteases (bromelain and papain). The primary objective was to evaluate vacuum impregnation (VI) and traditional marination (TM) techniques and assess their impact on beef's physicochemical attributes, shelf-life stability, and its plant protease activity. Shelf-life tests over 5 days revealed that all beef samples' pH reached its lowest on day 4, subsequently increasing on day 5. Throughout storage, colour parameters (L*, a*, b*, and ΔE) of treated samples consistently decreased. VI-PINE exhibited the lowest water holding capacity (WHC) over 5 days. Texture profile analysis (TPA) indicated that VI samples' hardness significantly reduced in VI-PINE and VI-PAPAYA compared to TM samples, attributed to coupled plant proteases. VI-PINE showed the highest marinade uptake improvement (21.6%); while having the lowest WHC (15.52%), and all samples' pH ranged in 5.15–6.12. The colour parameters of treated samples increased compared to raw untreated samples. Compared to TM, VI-PINE (1115 g) and VI-PAPAYA (1333 g) significantly reduced beef hardness, with VI-marinated beef also exhibiting higher tyrosine release compared to TM techniques. The application of VI on beef cubes with plant protease-containing marinades provides practical insights for the food industry, offering potential avenues to enhance beef quality, improve shelf life, and optimize flavour incorporation.

INTRODUCTION

Vacuum impregnation (VI) is a promising non-destructive food technology renowned for its capacity to facilitate solution diffusion into the food matrix, leading to improved physicochemical properties, sensory attributes, and prolonged shelf life of food products [1]. This two-step process involves eliminating trapped air through a vacuum and subsequently infusing desired solutions into food pores upon restoring atmospheric pressure. VI applications include reducing meat curing time and achieving uniform extracellular solution distribution in meat products through a hydrodynamic mechanism [1]. The experimental setup for VI is depicted in **Fig. 1**.

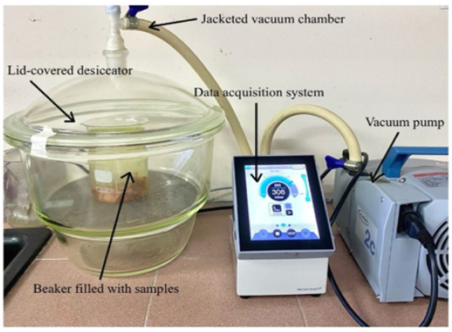

Fig. 1. Vacuum impregnation experimental device.

Home cooks often face prevalent challenges with beef toughness, hindering meal preparation to achieve desired meat texture. Natural tenderizers—specifically, pineapple juice (*Ananas comosus L.*) and raw green papaya juice (*Carica papaya L.*)—containing bromelain (EC 3.4.22.32) and papain (EC 3.4.22.2) respectively, were selected to address this issue. This study delves into the effectiveness of VI treatment with pineapple and papaya juice as meat tenderizers by comprehensively examining marinade uptake, pH, colour, water holding capacity (WHC), texture profile analysis (TPA), protease activity after marination, and evaluates 5-day shelf life, aiming to explore potential strategies for enhancing meat tenderness.

MATERIALS AND METHODS

Papaya (*Carica papaya*) and pineapple (*Ananas comosus*) from Pasar Borong Selangor was extracted by Panasonic Juicer MJ-70M-UB. Next, 50 mL fruit juices were diluted with 450 mL distilled water, create a 10% (v/v) marinade in a total volume of 500 mL. Fresh beef was uniformly cut into 2 cm × 2 cm × 2 cm dimensions as shown in **Fig. 2** and subjected to two marination techniques: traditional marination (TM) and vacuum impregnation (VI) according to **Table 1** and **Table 2**. VI process was executed using equipment from VACUUBRAND GMBH + CO KG in Wertheim, Germany. Three marinades were employed for both techniques: distilled water (CONTROL), pineapple (PINE), and papaya (PAPAYA).

Fig. 2. The uniform sizes of raw beef cubes.

Table 1. Traditional marination method parameters.

Beef Samples	Marination condition	
	Temperature (°C±1)	Duration (minutes)
RAW (unmarinated)	4	60
TM-CONTROL	4	60
TM-PINE	4	60
TM-PAPAYA	4	60

Table 2. Vacuum impregnation method parameters.

Beef Samples	Marination condition		
	Temperature (°C±1)	Pressure (mbar)	Duration (minutes)
VI-CONTROL	23	800	2
VI-PINE		600	2
VI-PAPAYA		400	2
		200	2
		75	15
		200	3
		400	3
		600	3
		800	3
		1000	25

Brix and pH values of the marinades were measured using Atago 3810 (PAL-1) Digital Pocket Refractometer and Jenway 3404 Bench pH meter respectively, both before and after adding distilled water. Marinade uptake of beef samples was determined using analytical balance Sartorius Extend series (Model ED124S) [2]. The pH and colour were measured using pH meter and colourimeter (Konica Minolta Chroma Meter CR-410) [3]. Water holding capacity (WHC) was assessed using centrifuge machine (Hettich Zentrifugen, Model EBA200S) [4]. Texture profile analysis (TPA) was conducted with texture analyzer (Stable Micro Systems Model TA-XT2i) [5]. Protease activity of bromelain and papain was evaluated using ThermoFisher Scientific, Genesys 10S UV-Vis spectrophotometer at 280 nm [6]. Shelf-life assessment was performed over five days, with physicochemical analyses in triplicate and daily photographs taken [7]. Analyses were done in triplicate and presented results in (mean ± standard deviation). TPA was repeated five times and expressed as (mean ± standard error of mean). Statistical analysis involved Two-way Analysis of Variance (ANOVA) with Tukey's tests at 95 % confidence level ($p<0.05$) using Minitab software.

RESULT AND DISCUSSION

Table 3 revealed that adding distilled water to pineapple and papaya juices slightly increased their pH but reduced Brix value. Both marinades' pH was within the active range for bromelain (pH 2.5-12) [8] and papain (pH 4.5-6.7) [9]. Dilution is crucial in vacuum impregnation (VI) processes to minimize Brix differences, enhance standardization, and address factors like solution types, viscosity, temperature, and solution-food ratio [10, 11].

Table 3. The pH and Brix values of different marinades.

Marinades	Enzyme	pH	Brix
Pineapple juice	Bromelain	3.80 0.02 ^{B}	9.33 0.06 ^{A}
Pineapple marinade	Bromelain	4.41 0.00 ^{A}	0.87 0.12 ^{B}
Green papaya juice	Papain	5.43 0.06 ^{b}	5.73 0.01 ^{a}
Green papaya marinade	Papain	5.98 0.00 ^{a}	0.70 0.00 ^{b}

[1]Data were expressed as mean ± standard deviation.
[2]Different uppercase letters indicate significant differences between pineapple juice and marinades.
[3]Different lowercase letters indicate significant differences between papaya juice and marinades.

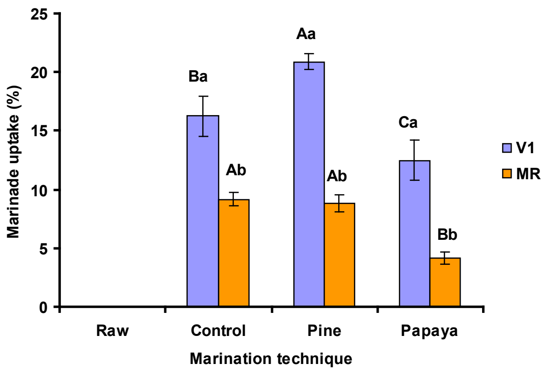

Fig. 3. Graph of marinade uptake (%) against the marination techniques. Data were expressed as mean ± standard deviation. Different uppercase letters indicate significant differences between marinades (Control, Pine & Papaya). Different lowercase letters indicate significant differences within the marination process (VI & TM).

JOBIMB, 2024, Vol 12, No 1 SP1, 78-85
https://doi.org/10.54987/jobimb.v12iSP1.949

Fig. 3 showed VI-treated meat significantly improved marinade uptake, outperforming traditional marination (TM) and RAW meat samples, corresponding with a similar study [12]. Vacuum forces fluid out of meat tissue, compressing residual gas upon atmospheric pressure restoration, maximizing marinade immersion into meat pores via capillary action [10, 11]. VI-PINE showed highest marinade uptake (21.07 % ± 0.63) due to high collagenolytic activity of bromelain which effectively breaking down myosin protein and increasing marinade absorption surface area [13, 14], while TM-CONTROL had the highest uptake among TM-treated meat due to its refrigerating temperature that cause enzyme inactivation [15].

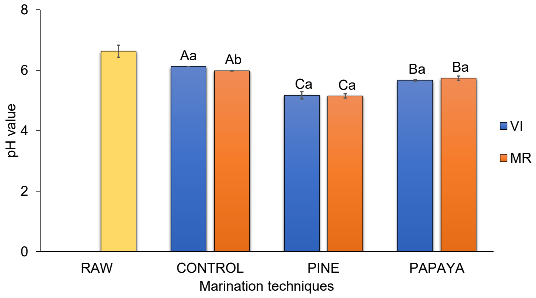

Fig. 4. Graph of pH against the marination techniques. Data were expressed as mean ± standard deviation. Different uppercase letters indicate significant differences between marinades (Control, Pine & Papaya). Different lowercase letters indicate significant differences within the marination process (VI & TM).

The pH of samples decreased after both VI and TM, with VI-PINE and TM-PINE having the lowest pH values ($p<0.05$) as shown in **Fig. 4**. This attributed to the acidic marination environment enhanced tenderness through increased collagen and myofibrillar solubility, causing muscle protein swelling [16]. No significant pH changes were observed between VI and TM techniques in papaya and pineapple marinades, like study [12], except for VI-CONTROL and TM-CONTROL which corresponds a previous study [17].

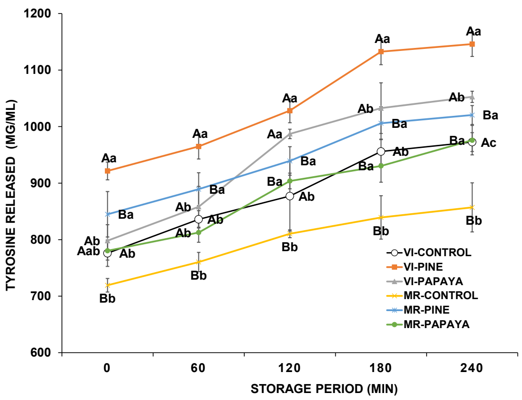

Fig. 5. Changes in pH of meat samples throughout the storage period. Data were expressed as mean ± standard deviation. Different uppercase letters indicate significant differences between marination techniques with same marinade (VI & TM). Different lowercase letters indicate significant differences between marinades within same marination techniques (DW, Pine & Papaya).

Fig. 5 depicted samples' pH decreased drastically on day-4, and increased on day-5, primarily due to lactic acid accumulation during glycogen conversion. VI had a pronounced impact on pH due to its higher diffusion rate and efficiency during meat penetration, facilitated by a controlled hydrodynamic mechanism (HDM), compared to TM.

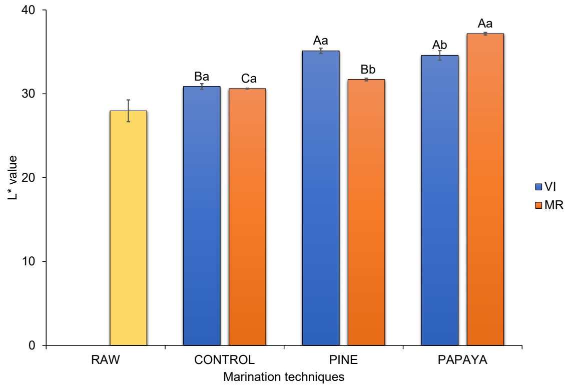

Fig. 6. Graph of L* against the marination techniques. Data were expressed as mean ± standard deviation. Different uppercase letters indicate significant differences between marinades (Control, Pine & Papaya). Different lowercase letters indicate significant differences within the marination process (VI & TM).

Fig. 6 demonstrated significant difference in L* value ($p<0.05$) between VI-PINE/TM-PINE and between VI-PAPAYA/TM-PAPAYA indicating variations in marination techniques. Moisture content positively influences L* value, suggesting VI resulted in more exuded water to impregnate meat surface [18]. TM techniques showed differences across marinades; but VI-PINE and VI-PAPAYA were correlated due to similar hydrolysis profiles of papain and bromelain [13].

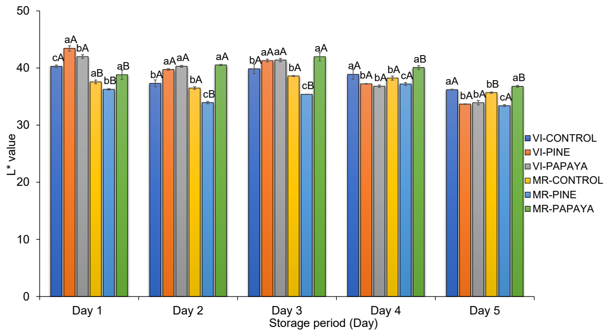

Fig. 7. Changes in L* value of meat samples throughout the storage period. Data were expressed as mean ± standard deviation. Different uppercase letters indicate significant differences between marination techniques with same marinade (VI & TM).3Different lowercase letters indicate significant differences between marinades within same marination techniques (Control, Pine & Papaya).

A gradual decrease was observed in L* values for all beef samples over 5 days as depicted in **Fig. 7**, same as study [19], with VI-PINE and VI-PAPAYA exhibiting a more pronounced reduction, while TM-PINE and TM-PAPAYA did not. Low pH marinades can induce denaturation in animal muscle proteins, impacting water binding capacity and light reflection, leading to lighter-coloured muscle formation [20].

JOBIMB, 2024, Vol 12, No 1 SP1, 78-85
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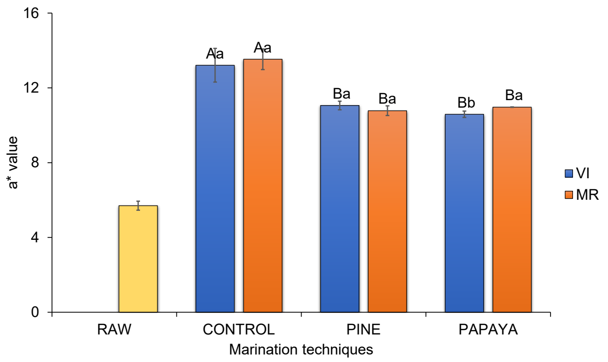


Fig. 8. Graph of a* value against the marination techniques. Data were expressed as mean ± standard deviation. Different uppercase letters indicate significant differences between marinades (Control, Pine & Papaya). Different lowercase letters indicate significant differences within the marination process (VI & TM).

Fig. 8 showed the highest a* value in both the CONTROL, consistent with comparable findings in chicken meat [21], but PINE and PAPAYA marinades were interconnected, indicating similar effects of bromelain and papain on beef redness.

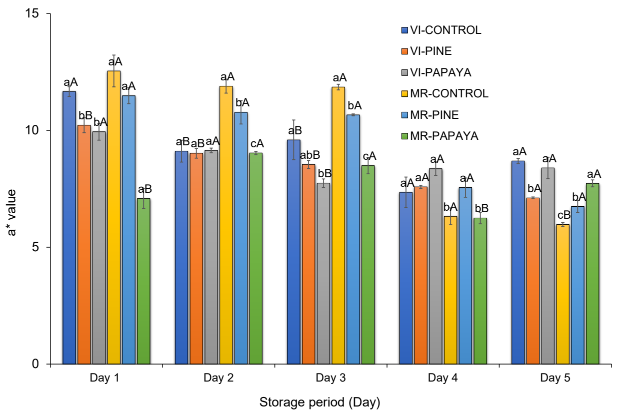


Fig. 9. Changes in a* value of meat samples throughout the storage period. Data were expressed as mean ± standard deviation. Different uppercase letters indicate significant differences between marination techniques with same marinade (VI & TM). Different lowercase letters indicate significant differences between marinades within same marination techniques (Control, Pine & Papaya).

PINE and PAPAYA marinated meat showed lower redness in meat compared to CONTROL in **Fig. 9**, due to their acidic pH and increased hydrogen ions, which accelerates the conversion of myoglobin to metmyoglobin, resulting in lower colour intensity [22].

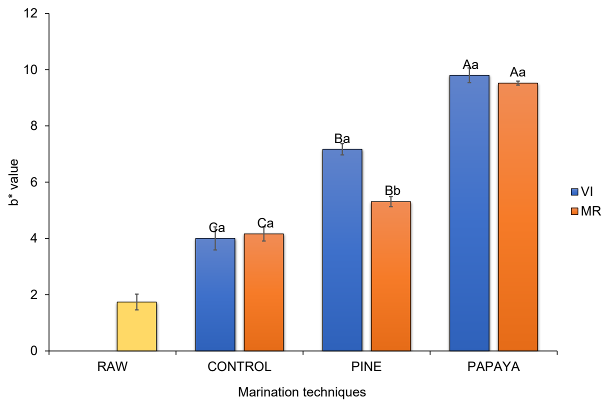


Fig. 10. Graph of b* value against the marination techniques. 1Data were expressed as mean ± standard deviation. Different uppercase letters indicate significant differences between marinades (Control, Pine & Papaya). Different lowercase letters indicate significant differences within the marination process (VI & TM).

Fig. 10 showed differences in beef samples' yellowness across marinade types ($p<0.05$), with CONTROL having the lowest b* value, aligned with findings [23]. Compared to TM-PINE, VI-PINE could penetrate deeper into capillary pores of meat due to VI.

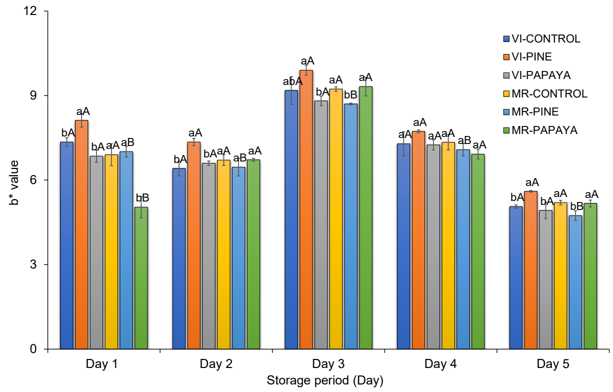
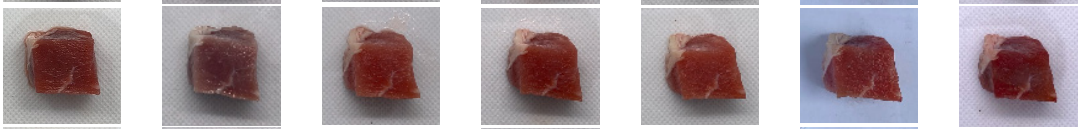
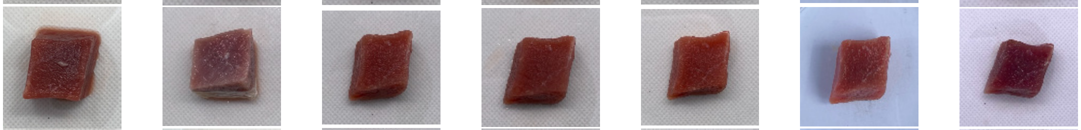


Fig. 11. Changes in b* value of meat samples throughout the storage period. Data were expressed as mean ± standard deviation. Different uppercase letters indicate significant differences between marination techniques with same marinade (VI & TM). Different lowercase letters indicate significant differences between marinades within same marination techniques (Control, Pine & Papaya).

No significant differences found between TM and VI in **Fig. 11**, except VI-PINE and TM-PINE, with VI-PINE maintaining the highest b* value throughout storage due to pineapple marinade's yellow pigments.

Table 4. Visual changes of beef samples under different marination techniques during storage.

Marination Techniques	Day 0 (Before Marination)	Day 0 (After Marination)	Day 1	Day 2	Day 3	Day 4	Day 5
RAW							
VI-PINE							
VI-PAPAYA							
VI-CONTROL							
TM-PINE							
TM-PAPAYA							
TM-CONTROL							

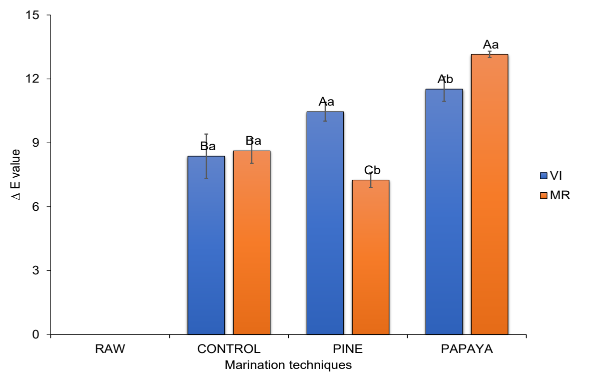


Fig. 12. Graph of ΔE value against the marination techniques. Data were expressed as mean ± standard deviation. Different uppercase letters indicate significant differences between marinades (Control, Pine & Papaya). Different lowercase letters indicate significant differences within the marination process (VI & TM).

Apparently, **Fig. 12** showed that marination techniques significantly influence on ΔE values, VI-PINE and VI-PAPAYA effectively induces colour changes in beef, with positive correlation observed between VI-PINE and VI-PAPAYA, while VI-CONTROL resulting in the least dynamic colour changes.

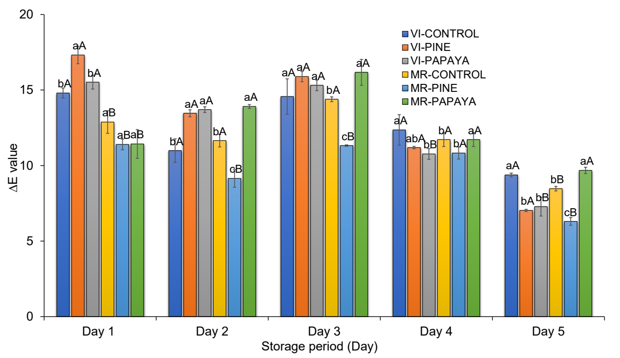


Fig. 13. Changes in ΔE value of meat samples throughout the storage period. Data were expressed as mean ± standard deviation. Different uppercase letters indicate significant differences between marination techniques with same marinade (VI & TM). Different lowercase letters indicate significant differences between marinades within same marination techniques (Control, Pine & Papaya).

Fig. 13 demonstrated TM-PAPAYA deviated from the consistent pattern of decreasing ΔE values for treated samples, exhibiting an increase, subsequent decline, and continuing decrease. Overall, lipid oxidation leads to the overall ΔE reduction, which in turn causes marinated meat discolouration over time [15].

JOBIMB, 2024, Vol 12, No 1 SP1, 78-85
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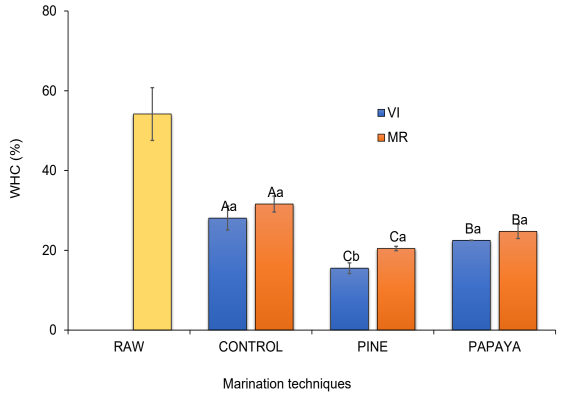

Fig. 14. Graph of water holding capacity (WHC) against the marination techniques. Data were expressed as mean ± standard deviation. Different uppercase letters indicate significant differences between marinades (Control, Pine & Papaya). Different lowercase letters indicate significant differences within the marination process (VI & TM).

Marinade types significantly affect meat WHC ($p<0.05$) as shown in **Fig. 14**. CONTROL marinades exhibited the highest WHC, while pineapple and papaya marinades decreased WHC due to plant enzymes hydrolyzing meat proteins, causing shrinkage in meat fibres, gel formation in myofibrillar proteins, membrane destruction [24]. Raw meat's high WHC (54.17 %) is due to its pH 6.63, near major meat proteins' isoelectric point. TM-meat showed higher WHC than VI-meat due to VI process damage and water loss, contrasting with studies [25] suggesting VI can improve WHC via actomyosin dissociation, possibly influenced by experimental conditions.

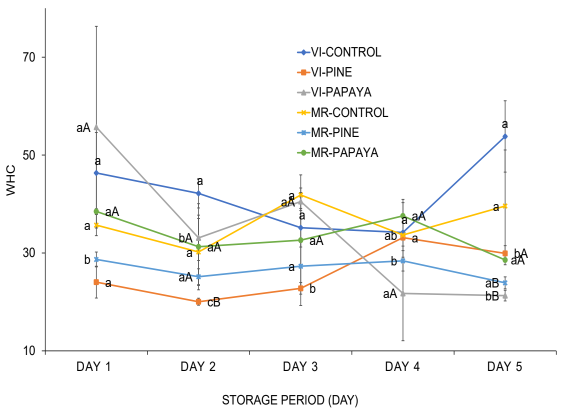

Fig. 14. Changes in WHC of meat samples throughout the storage period. 1Data were expressed as mean ± standard deviation. Different uppercase letters indicate significant differences between marination techniques with same marinade (VI & TM). Different lowercase letters indicate significant differences between marinades within same marination techniques (Control, Pine & Papaya)

No discernible WHC pattern over storage study, possibly due to factors like chilling, handling method, measurement duration, and meat sizes that complicating interpretation [26, 27].

Table 5. Textural properties of treated and untreated beef.

Treatment /Marinades	Hardness (g)	Adhesiveness (g.sec)	Springiness	Chewiness
RAW	3856 ± 1509.1	-18.88 ± 6.96	0.4936 ± 0.03	1012 ± 440.24
VI-CONTROL	2754 ± 511.90	-13.76 ± 2.09	0.4348 ± 0.05	798 ± 279.25
VI-PINE	1115 ± 163.72	-21.68 ± 2.28	0.4536 ± 0.02	189.6 ± 27.35
VI-PAPAYA	1333 ± 244.79	-22.07 ± 5.71	0.6268 ± 0.10	352 ± 158.94
TM-CONTROL	2899 ± 420.32	-23.20 ± 4.39	0.5014 ± 0.04^{AB}	364 ± 131.34
TM-PINE	1263 ± 126.68	-19.25 ± 3.16	0.4052 ± 0.03^{B}	200.4 ± 43.29
TM-PAPAYA	1675 ± 188.76	-29.05 ± 5.10	0.596 ± 0.02^{A}	422.5 ± 98.55

[1]Data were expressed as mean ± SEM.
[2]Different uppercase letters indicate significant differences between marinade types (Control, Pine, Papaya).

Table 5 demonstrated that VI technique significantly reduced meat hardness compared to TM attributed to microstructural alterations in meat fibres. When VI coupled with plant proteases, VI-PINE results in more pronounced decrease in meat hardness. VI enhances meat tenderness by uniformly distributing marinade across the meat, facilitating protein hydrolysis to disrupt connective tissue structure. However, no significant differences were found in adhesiveness, springiness, or chewiness, suggesting VI on meat chewiness is linked to diminished protein matrix WHC [12].

Table 6 showed that VI-PINE and VI-PAPAYA effectively softened meat, with VI-CONTROL showing lower hardness. TM-PAPAYA initially demonstrated higher hardness, but no significant differences between VI and TM over time. Both VI-PINE and TM-PINE showed low adhesiveness and high springiness due to bromelain-induced protein hydrolysis, which also reduces shear force and hardness [28]. Chewiness in all samples decreased over time, likely due to protein hydrolysis weakening bonds [29].

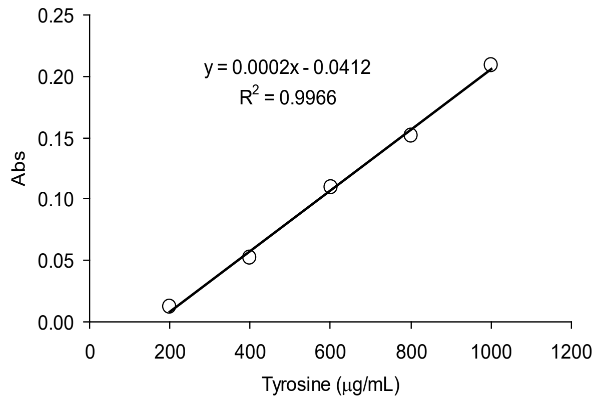

Fig. 15. Tyrosine standard calibration curve.

Table 6. The textural profile changes throughout the storage period.

Sample / Attribute	DAY	VI			TM		
		CONTROL	PINE	PAPAYA	CONTROL	PINE	PAPAYA
Hardness (g)	1	2162 ± 1000	1451 ± 40	729 ± 100	2683 ± 1119	2067 ± 636	3889 ± 475
	2	5650 ± 2369	2094 ± 740	1156 ± 456	3010 ± 1411	1027 ± 393	4290 ± 1380
	3	2038 ± 148^{ab}	610 ± 217^{b}	2177 ± 519^{a}	2504 ± 357	449 ± 88	1820 ± 406
	4	3107 ± 313^{aA}	1385 ± 162^{b}	1900 ± 280^{b}	1641 ± 104^{B}	1374 ± 301	2375 ± 876
	5	1964 ± 780	1019 ± 268	637 ± 141	983 ± 225	780 ± 388	802 ± 139
Adhesiveness (g.sec)	1	-8.95 ± 3.62^{a}	-58.05 ± 0.76^{bA}	-15.60 ± 3.89^{a}	-21.02 ± 9.09^{a}	-38.80 ± 3.65^{aB}	-16.30 ± 3.69^{a}
	2	-18.1 ± 10.59^{a}	-76.1 ± 11.06^{b}	-7.77 ± 2.99^{aA}	-9.73 ± 2.81^{a}	-57.66 ± 5.08^{b}	-26.53 ± 4.58^{aB}
	3	-31.9 ± 10.81^{ab}	-46.35 ± 4.70^{b}	-8.43 ± 2.88^{a}	-24.68 ± 9.08^{a}	-43.8 ± 12.15^{a}	-19.58 ± 2.86^{a}
	4	-25.25 ± 3.34^{aB}	-89.75 ± 7.83^{b}	-12.33 ± 0.88^{a}	-7.82 ± 2.67^{aA}	-93.9 ± 12.56^{b}	13.03 ± 3.53^{a}
	5	-18.97 ± 4.41^{a}	-46.5 ± 22.16^{a}	-23.85 ± 8.55^{a}	-10.27 ± 1.13^{a}	-48.43 ± 5.29^{b}	15.59 ± 0.19^{a}
Springiness (mm)	1	0.53 ± 0.00^{bA}	0.89 ± 0.01^{a}	0.42 ± 0.06^{b}	0.49 ± 0.01^{aB}	0.76 ± 0.11^{a}	0.50 ± 0.02^{a}
	2	0.54 ± 0.02^{b}	0.86 ± 0.07^{a}	0.50 ± 0.05^{b}	0.48 ± 0.04^{b}	0.92 ± 0.03^{a}	0.54 ± 0.03^{b}
	3	0.55 ± 0.03^{b}	0.90 ± 0.01^{a}	0.38 ± 0.02^{cB}	0.56 ± 0.02^{b}	0.88 ± 0.07^{a}	0.61 ± 0.02^{bA}
	4	0.48 ± 0.01^{b}	0.92 ± 0.01^{aA}	0.52 ± 0.02^{b}	0.44 ± 0.02^{b}	0.85 ± 0.01^{aB}	0.51 ± 0.06^{b}
	5	0.58 ± 0.11^{a}	0.65 ± 0.16^{a}	0.69 ± 0.13^{a}	0.54 ± 0.03^{b}	0.95 ± 0.03^{a}	0.59 ± 0.05^{b}
Chewiness	1	123.43 ± 1.81^{b}	1048 ± 175.10^{aA}	448 ± 207.80^{ab}	548 ± 298.66	218.80 ± 1.08^{B}	829 ± 21.14
	2	1679 ± 833.26	848 ± 329.05	250 ± 105.36	815 ± 462.58	456 ± 218.91	1318 ± 584.22
	3	442.4 ± 90.07	286 ± 101.17	335 ± 105.30	638 ± 85.04	189 ± 32.86	580 ± 181.41
	4	689 ± 134.11	534.2 ± 54.49	449.2 ± 89.61	336.8 ± 27.78	552.2 ± 60.86	501 ± 164.63
	5	450 ± 155.42	259 ± 108.94	184.3 ± 17.79	268.2 ± 74.04	321 ± 145.77	222.8 ± 59.99

[1]Data were expressed as mean ± SEM.
[2]Different uppercase letters indicate significant differences between marination techniques with same marinade (VI & TM).
[3]Different lowercase letters indicate significant differences between marinades within same marination techniques (CONTROL, PINE & PAPAYA)

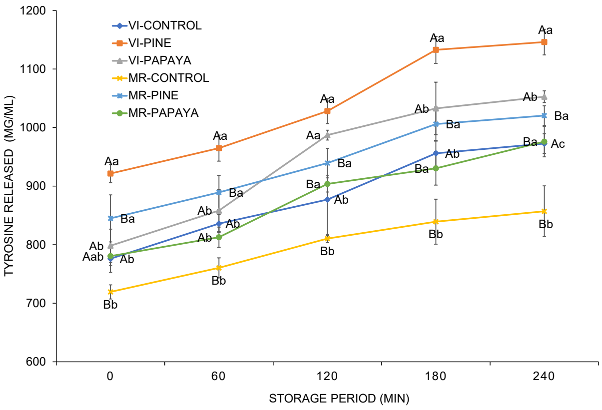

Fig. 16. Changes in tyrosine released of meat samples throughout the storage period. Data were expressed as mean ± standard deviation. Different uppercase letters indicate significant differences between marination techniques with same marinade (VI & TM). Different lowercase letters indicate significant differences between marinades within same marination techniques (Control, Pine & Papaya).

A tyrosine standard calibration curve was plotted in **Fig. 16** to measure tyrosine liberated during proteolytic activity. **Fig. 17** revealed that VI coupled with plant proteases had higher efficacy as it released tyrosine more rapidly than TM techniques. Bromelain and papain aid in rapid meat tenderization through enzymatic hydrolysis, targeting collagen and myofibrillar proteins [29]. Increased mass transfer rates in VI, influenced by tissue porosity and shape [11], expedited external solution infusion, thereby demonstrating effective enzymatic hydrolysis for enhanced meat tenderness.

CONCLUSION

In summary, this study explores the effects of vacuum impregnation (VI) and traditional marination (TM) on beef quality, finding that when VI is integrated with proteolytic enzymes, it improves pH regulation, colour enhancement, and tenderness, addressing concerns of meat toughness. Both VI and TM improved beef colour, texture and tenderness, with pineapple marinades enhancing palatability and minimizing hardness, while papaya marinades showed efficacy with varying outcomes. Results depend on factors like cattle species, diet, and storage conditions.

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