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Effects of different concentrations of pineapple core extract and maceration process on free-range chicken meat quality

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PAPER

Abstract

The tenderisation effects of pineapple (Josephine variety) core extracts on the quality of free-range chicken meat using different maceration concentrations (30%, 50%, and 100%) and duration (15, 25, and 35 min) were analysed. Texture profile analysis, colour, pH, bromelain content, and microbiological analyses of the macerated meat samples were assessed. Broiler breast meat macerated with core extract (100%, 35 min) showed 86% reduction in hardness and the pH decreased from 5.87 to 4.99. The pineapple core extract has great potential as a meat tenderiser thus reduces the agriculture by-product and converts it into natural food ingredients.

Keywords: bromelain content; broiler breast; maceration; meat quality; pineapple core extract; tenderisation process

Introduction

Increased export capacity and processing of pineapple are estimated to reach RM320 billion. In Malaysia, the pineapple plantation covering 13,433 hectares of farmland with a yield of about 32.37 tonnes of pineapple per hectare produce a total volume of 434,811 metric tonnes (Nor Mazila, 2020). This mass production generates a substantial by-product consisting of residual pulp, leaf, stem, peels, cores, crowns high in sugar, pectin (insoluble fibre), crude fibre, and proteins. The increasing volume of waste is detrimental to health because pineapple waste takes quite a long time to degrade and attract pests, leading to an increased risk of various dangerous diseases. Thus, there is a need to convert this core into a value-added commodity. It consists of a sizeable amount of antioxidant property, sugar, fibre, vitamin C, protein, phenolic compound, carotenoids, and flavonoids (Hanafi and Abdullah, 2008). Pineapple core waste contains a high amount of bromelain enzyme, widely used in the food industry for tenderising meat (Janhvi *et al*., 2016), chill proofing beer (Ketnawa and Rawdkuen, 2011), leather tanning process, in latex manufacturing (Christner *et al*.,1992), skincare products (Frank and Schulze, 2010), and pharmaceuticals (Bhattacharyya, 2008).

Bromelain acts on meat by breaking down the collagen fibres and shows hydrolytic activity on the connective tissue, leading to the tenderisation of meat. The bromelain action on meat is affected by various factors such as pH, water-holding capacity, moisture content, and concentration (Janhvi *et al*., 2016). A method to tenderise the tough meat is essential to increase its acceptability. According to Gok and Bor (2016), the typical way to tenderise meat is by the maceration technique. The maceration time may vary according to the type of marinade used to over-tenderise the meat surface, leading to undesirable "mushy" meat (Han *et al*., 2009).

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Therefore, this study aims to determine the effect of using different concentration core extracts and maceration durations on free-range chicken meat quality. According to the United States Department of Agriculture (USDA), free-range-chicken means that the chicken has full access and freedom to roam outdoors, outside of their pens, at any given time. The pineapple core by product of Josephine in this study could be considered as a potential meat tenderiser for a broiler chicken quality, commonly associated with tough and rigid texture among local people.

Materials and Methods

Preparation of pineapple core extract

Josephine pineapple was purchased from the New Seng Kee (NSK) hypermarket located at Jalan Peel, Kuala Lumpur, Malaysia. The basis of selection was the size (1.2–1.5 kg), firmness (C3 maturation stage), and skin colour (yellow/orange on two thirds), with no secretion from the skin. These parameters were measured visually using the naked eye (Yuris and Lee, 2014). The pineapples were washed thoroughly using tap water, cored, and then weighed (Mettler Toledo, US). The pineapples' cores were extracted out and subjected to the juicing process using a juicer (Panasonic, Malaysia), filtered using muslin cloth with a mesh size of 2 mm, and stored in a sterile bottle. The yield after the extraction of 20 cores was about 1000 mL of extract. The extracted juice was kept at 4°C before the maceration treatment.

Proximate analysis, pH, and total soluble solid analysis of pineapple juice core extract

Following proximate analysis, ash, moisture content, and crude fibre were determined by using the standard AOAC Method 2006. The pH value was recorded using a digital pH meter (PT-15, Sartorius, Germany). Total soluble solids (TSS) in the core extract were determined using a handheld analog refractometer (0-32°) (Atago, Japan), and the results were expressed as per cent soluble solids (° Brix)

Bromelain content analysis

The enzyme activity of fresh pineapple core and macerated broiler breast meat was determined according to the casein digestion method and tyrosine standard (Edmund and Isaac, 2018). The assay mixture contained 5 mL of freshly prepared 1% casein, which was pre-warmed at 37°C, to be used as substrate and 1 mL of the freshly prepared solubilised bromelain was added in the mixture. The mixture was vortexed immediately and incubated at 37°C for 10 min. The reaction was stopped by the addition of 5 mL of 1% Trichloroacetic acid. The reaction mixture was filtered and the absorbance of the filtrate was measured at 280 nm using a spectrophotometer. Using tyrosine as a standard, concentrations of 50 µg/mL, 100 µg/mL, 150 µg/mL, 200 µg/mL, and 250 µg/mL were prepared, and their absorbance read at 280 nm. A standard curve of tyrosine absorbance (Y axis) against tyrosine concentration (X axis) was plotted, refer to equation (1):

$$\text{Activity(CDU / mL)} = \frac{\text{Et} - \text{Eb}}{\text{Es}} \text{Concentration of Standard L} - \text{tyrosine} \times \frac{\text{Vr}}{\text{tr}} \times \text{Df}, \quad (1)$$

where

CDU = casein digestion unit
Et = absorbance of enzyme sample
Df = dilution factor
Eb = absorbance of enzyme blank
Vr = reaction volume
Es = absorbance of standard l-tyrosine
tr = reaction time

Browning inhibition in pineapple core extract juice

A low browning inhibition value indicates a high level of browning in the core, as described by Kim *et al.* (2005) The absorbance was read at 420 nm for every 45 min using a UV-VIS Spectrophotometer (UV-1900, Shimadzu, Japan). The browning inhibition percentages were calculated using equation (2):

$$\text{Inhibition (\%)} = [(\text{AF}_{\text{blank}} - \text{AI}_{\text{blank}}) - (\text{AF}_{\text{sample}} - \text{AI}_{\text{sample}}) \times 100] / \text{A}_{\text{blank}}, \quad (2)$$

where

$\text{AF}_{\text{sample}}$ is the final absorption of the sample

AF_{blank} is the final absorption of the control

AI_{blank} is the initial absorption of the control

Maceration process for tenderisation effect in broiler breast meat

The broiler cuts from the breast part were selected and purchased from the local market in Pudu, Selangor, Malaysia. The breast meat was chosen, as it displays a firmer, more rigid, and thicker texture than the other

broiler chicken meat parts (Debora *et al*., 2017). The visible fats and connective tissue were removed before maceration. The maceration procedure of the meat was performed, as described by Bhaskar *et al*. (2007). Meats were manually cut into uniform size, approximately 2 × 2 × 2 cm before further analysis. The meat and pineapple core extract ratio was maintained as 1:1 (meat: marinade) (Gok and Bor, 2016). The broiler meat chunks were macerated in the core extract for 15, 25, and 35 min at three different concentrations: 100% (1:0), which contains 250 mL of the whole core extract/without dilution; 50% (1:1), which contains 125 mL of the core extract and 125 mL of distilled water; and 30% (1:2), which contains 75 mL of the core extract diluted with 175 mL of distilled water, respectively, to obtain the maximum effect of up to 250 mL of the total volume. Meat samples were macerated at room temperature (27 ± 3°C) and placed in a zip lock bag during the maceration process. Fresh meat samples macerated using distilled water was used as a control.

Texture profile analysis of macerated broiler breast meat

Texture profile analysis was carried out using the texture analyser (Stable Micro System, UK) with a flat-ended cylindrical probe. The test samples were compressed to 50% of their original height with the setting of 1.0 mm/s, 4.2 mm/s, and 5.0 mm/s for pre-set speed, test speed, and post-test speed, respectively. The textural parameters of the meat were hardness and chewiness (Nadzirah *et al*., 2016). All the analyses were performed in triplicate at room temperature of 27°C, and the mean and standard deviation (SD) were calculated.

Colour analysis

Colour measurement was done on the surface of the macerated meat samples by using Chroma Meter (CR-410, Konica Minolta, Japan). The illuminant D65 (representing typical daylight) was used during analysis. The L* (lightness), a* (redness), b* (yellowness) values of meat samples were measured and calculated. The average value of three meat samples for each maceration duration and concentration (triplicates) were used for statistical analysis (Nadzirah *et al*., 2016).

Microbiological analysis

The meat samples' microbial load after each maceration duration was measured using the Total Plate Count Method (AOAC 966.23). After solidifying the nutrient agar (Merk, Darmstadt, Germany), the plates were inverted and incubated for 48 h at 37°C. The entire colony-forming units (CFU) were counted, including those of pinpoint size. The parameter used to count the colonies: regular plates (25–250 counts), plates with more than 250 colonies for all dilutions (too many to count), and plates with less than 25 colonies for all dilutions (too low to count).

Statistical analysis

All results were expressed as the mean ± SD. The results obtained were analysed with a two-way ANOVA using Minitab version 17 statistical package to determine if there was any significant difference between maceration duration and concentration of the core extract with regard to meat quality. Turkey's method was used to determine which pair is significantly different from each other. Differences were significant when $P < 0.05$.

Results and Discussion

Pineapple core extract characteristics

The proximate analysis of pineapple core extract revealed the moisture content, ash content, protein content, crude fibre, pH, TSS, and enzyme properties (i.e., browning inhibition and bromelain activity) of the Josephine variety of pineapple core extract, as shown in Table 1.

The pH of macerated broiler breast meat

The pH values of the control and macerated meat samples are shown in Table 2. It can be observed that the pH value of the control meat samples was the highest compared to the treated meat samples. The treated meat samples showed a significant decrease in pH value for all concentrations when the maceration duration increased. However, the pH value significantly increased when the concentration of the core extract decreased. It can be suggested that the lowest pH in broiler breast meat

Table 1. Physicochemical properties of pineapple core extract.

Analysis	Josephine pineapple core extract value
Moisture content (%)	89.67 ± 0.24
Ash content (%)	0.75 ± 0.04
Protein content (%)	19.68 ± 0.09
Crude fibre (%)	1.74 ± 0.14
pH	3.83 ± 0.01
Total soluble solid (°Brix)	9.60 ± 0.00
Browning inhibition (%)	4.18 ± 0.25
Bromelain activity (CDU/mL)	152.01 ± 1.54

is influenced by the acidic pH (3.83 ± 0.01) of the core extract. These findings are similar to those of Mohamad Afifi *et al*. (2018), who reported that the lowest pH (5.61) of the beef cut sample is most likely due to the pH of the core extract and maceration time.

According to Ketnawa and Rawduken (2011), pineapple juice, which contains bromelain, will hydrolyse the muscle, thus releasing amino acid to reduce the meat's pH. These findings were supported by Manohar *et al*. (2016) who reported that an increase in the pineapple extract concentration decreases the treated boneless meat samples' pH, thus becoming more acidic. Maryana *et al*. (2018) reported that any pH range treatments from 4 to 5 could decrease meat texture's hardness. Any pH between the isoelectric point of myofibrillar protein reduced the capacity to bind water. Burke and Monahan (2003) also reported a significant reduction in pH from 5.7 to 3.1 of bovine muscle strips marinated with citrus juice compared to the untreated samples. Thus, the meat's acidity can be used as an indicator to detect the soft meat texture. The lower the pH value, the greater the meat tenderisation effect (Manohar *et al*., 2016). The broiler breast meat macerated in 100% concentration of Josephine pineapple core extract for 35 min had the lowest pH value and significant tenderness compared to other treatments.

Bromelain content of the macerated broiler breast meat

The bromelain activity of the diluted core extract and the macerated meat's bromelain activity are shown in Table 3. It can be observed that when the concentration of the core extract decreased from 100% to 30%, the bromelain activity also decreased from 151.06 CDU/mL to 56.87 GDU/mL. It can be suggested that the bromelain activity was affected by the percentage of the concentrated core extract. Pineapple core, which has been regarded as a significant waste from pineapple production, was reported to have more bromelain content than other residue parts (Banerjee *et al*., 2020). These findings agreed with the findings of this study where the higher the concentrations of the pineapple core extract in the macerated meat, the higher the bromelain activity. Hence, with the increased maceration duration, higher tenderisation affects the meat, as shown in Table 4. The bromelain intervention has been proven by its potential to disrupt the muscle microstructure and cause myofibril protein degradation (Bhat *et al*., 2018).

Table 2. pH value of macerated broiler breast meat at different concentrations and durations.

Treatments (%)	Maceration duration (min)		
	15 min	25 min	35 min
Control (DW)	5.87 ± 0.02^{Aa}	5.87 ± 0.02^{Aa}	5.87 ± 0.02^{Aa}
Josephine (30)	5.65 ± 0.01^{ABa}	5.50 ± 0.01^{Bb}	5.54 ± 0.01^{Bb}
Josephine (50)	5.33 ± 0.01^{Ba}	5.20 ± 0.02^{Cb}	5.12 ± 0.01^{Cc}
Josephine (100)	5.30 ± 0.01^{Ba}	5.16 ± 0.01^{Cb}	4.99 ± 0.01^{Dc}

Values are expressed as mean ± standard deviation (n = 3).
Mean with different superscript capital letters within column are significantly different (P < 0.05).
Mean with different superscript lower case letters within row are significantly different (P < 0.05).
Notes: 100%, 50%, and 30%, concentration of the core extract; DW, distilled water; 15, 25, and 35 min, maceration duration.

Texture profile analysis

Texture profile analysis measured hardness and chewiness properties in meat to reflect the effect of bromelain enzyme as a natural meat tenderiser. The macerated meat's hardness and chewiness had significantly reduced (P < 0.05) with the extract's increased duration and concentration (Table 4). The longer maceration duration in meat reduced the hardness (3766.50 to 1044.20 N) and chewiness (4012.95 to 726.65 N) of the macerated meat. These findings align with the study carried out by Ketnawa and Rawdkuen (2011). They reported that by increasing the concentration of the bromelain extracted from the pineapple peel, a continuous decrease of hardness was found in marinated beef, chicken, and squid samples. A previous study by Daniela *et al*. (2012) observed that an increase in the meat treatments' action time leads to a significant increase in rigidity index value, reflecting the degree of tenderness of the meat. Based on the results, chicken meat macerated with 100% Josephine pineapple core extract for 35 min was significantly (P < 0.05) softer in texture than chicken meat subjected to other treatment concentrations and maceration durations. It shows the lowest value of hardness and chewiness compared to the other treatments.

Ketnawa and Rawdkuen (2011) reported that the increase in treated meat's tenderness was due to proteolysis enzyme action on myofibrillar protein by bromelain. The myofibrillar protein breakdown generates small peptides or protein with low molecular weight, thus increasing the meat samples' tenderness. Bille and Taapopi (2008) also found that bromelain's action in denaturing the protein and breaking down the collagen, muscle fibre, and tissue resulted in increased meat tenderness in their study samples of goat meat (back, ribs, and rear limbs). The samples were marinated with bromelain extract powder and citric marinade to tenderise for 10 min at room temperature. Rawdkuen and Benjakul (2012) also reported that the enzymes increased the collagen solubility, and this

Table 3. Bromelain content of the diluted pineapple core extract, macerated broiler breast meat at different concentrations and durations.

Concentration (%)	Bromelain activity (CDU/mL)			
	Diluted pineapple extract	Maceration duration (min)		
		15 min	25 min	35 min
Control (DW)	NA	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}	0.00 ± 0.00^{Aa}
Josephine (30)	56.87 ± 1.26^{A}	8.95 ± 0.01^{Ba}	12.93 ± 0.01^{Bb}	17.58 ± 0.01^{Bbc}
Josephine (50)	79.29 ± 1.01^{B}	12.28 ± 0.01^{Ca}	20.67 ± 0.02^{Cb}	29.49 ± 0.01^{Cc}
Josephine (100)	151.06 ± 1.11^{C}	18.30 ± 0.01^{Da}	26.47 ± 0.01^{Db}	39.19 ± 0.01^{Dc}

Values are expressed as mean ± standard deviation (n = 3).
Mean with different superscript capital letters within column are significantly different ($P < 0.05$).
Mean with different superscript lower case letters within row are significantly different ($P < 0.05$).
Notes: 100%, 50%, and 30%, concentration of the core extract; DW, distilled water; 15 , 25 , and 35 min, maceration duration; NA, not available.

promoted the structural alteration through the process of collagens cross-link.

Meat colour analysis

The control and treated meat samples' colour parameters for 15, 25, and 35 min are shown in Table 5 (L*, a*, and b*value). The results show that the maceration process significantly affected the L* and b* values of the broiler breast samples. The lightness (L*value) increased as the maceration duration increased but decreased when the core extract concentration decreased compared to the control. According to Kim *et al.* (2012), the meat colour influenced meat quality; hence, they were affected by marination.

The colour of the meat is related to muscle pigments, myoglobin, and haemoglobin. However, meat's discolouration is affected by pigment conditions (amount and chemical state). The entire breast muscle, commonly discoloured as breast muscle, comprises a large portion of the weight (~5%), so it is more sensitive, contributing to discolouration and the meat's light appearance.

Therefore, small changes in colour on the breast part is more noticeable than in other parts. Serdaroglu *et al.* (2007) reported that the increase in lightness is due to the swell of the muscle protein and light reflection altered at low pH and ionic strength, thus forming the lighter colour. According to Wismer-Pedersen (1959), it is widely accepted that variations in muscle structure may affect light reflectance or light scattering. The extent of denaturation of the muscle proteins differs in ordinary and pale coloured meat. The b*value decreased with increased maceration duration. However, when the concentration of the core extract decreased, the b*value increased. Meanwhile, the a*value of the control and treated meat samples shows no significant difference at all concentrations used during the first 15 min. A similar pattern of a*value was also reported by Serdaroglu *et al.* (2007) in which the turkey breast was marinated in grapefruit juice (50% and 100%) and citric acid (0.05 M, 0.1 M, and 0.2 M). The a*value (redness) is related to the concentration of myoglobin and myoglobin denaturation level (Francis and Clydesdale, 2008; Vaudagna *et al.*, 2008). The acid treatment appeared to enhance myoglobin's conversion to metmyoglobin, which results in lower colour intensity. Table 7 shows the colour difference (ΔE*) of macerated meat in different core extract concentrations at 15, 25, and 35 min. The highest colour difference was found in meat samples macerated in 100% concentration of Josephine core extracts for 35 min. According to Francis and Clydesdale (2008), when colour differences (ΔE*) exceed the value of 3, the meat's colour change is detectable to the human eye.

Microbiological analysis of the macerated broiler breast meat

According to Table 6, the total microbial count decreased as the maceration duration increased for all treatments. However, when the concentration of the core extract decreased, the total microbial count increased. In this study, the total microbial count was below 7 Log CFU/g after 35 min of maceration. In contrast, the highest microbial counts were observed as early as 15 min of maceration using 30% concentration of core extract (4.34 Log CFU/g) regardless of the control sample. According to Hong *et al.* (2013), this might be due to the sample's initial microbial contamination because bacterial counts in fresh meat are generally less than 3 Log CFU/g.

Jeong *et al.* (2018) also reported that citrus juice such as pineapple juice has an antimicrobial function, causes denaturation of microorganisms, and affects the water-holding capacity. The pH reduction caused by this

Table 4. Hardness and chewiness value of the macerated broiler breast meat at different concentrations and durations.

Treatments (%)	Texture profile analysis (TPA)					
	Hardness (*N*)			Chewiness (*N*)		
	15 min	25 min	35 min	15 min	25 min	35 min
Control (DW)	7292.30 ± 99.48^{Aa}	7292.30 ± 99.48^{Aa}	7292.30 ± 99.48^{Aa}	4012.95 ± 96.75^{Aa}	4012.95 ± 96.75^{Aa}	4012.95 ± 96.75^{Aa}
Josephine (30)	3766.50 ± 84.40^{Ba}	2755.60 ± 104.47^{Bb}	2741.80 ± 91.78^{Bb}	1711.87 ± 103.79^{Ba}	1281.10 ± 85.94^{Bb}	800.15 ± 96.87^{Bc}
Josephine (50)	2552.40 ± 92.25^{Ca}	2272.40 ± 91.97^{Cb}	1707.90 ± 85.78^{Cc}	1594.06 ± 87.25^{Ca}	1146.83 ± 101.79^{Cb}	733.46 ± 97.18^{BCc}
Josephine (100)	2224.90 ± 94.57^{Da}	1806.10 ± 89.05^{Db}	1044.20 ± 106.95^{Dc}	1107.22 ± 87.79^{Da}	1074.90 ± 91.82^{CDab}	726.65 ± 94.85^{Cb}

Values are expressed as mean ± standard deviation (n = 3).
Mean with different superscript capital letters within column are significantly different (P < 0.05).
Mean with different superscript lower case letters within the row are significantly different (P < 0.05).
Notes: 100%, 50%, and 30%, concentration of the core extract; DW, distilled water; 15, 25 and 35 min, maceration duration.

Table 5. Colour (L*, a*, b*value) of macerated broiler breast meat at different concentrations and durations.

Treatments (%)	Colour Analysis								
	L*value (lightness)			a*value (redness)			b*value (yellowness)		
	15 min	25 min	35 min	15 min	25 min	35 min	15 min	25 min	35 min
Control (DW)	40.49 ± 0.40^{Aa}	40.49 ± 0.40^{Aa}	40.49 ± 0.40^{Aa}	8.08 ± 0.30^{Aa}	8.08 ± 0.30^{Aa}	8.08 ± 0.30^{Aa}	12.59 ± 0.25^{Aa}	12.59 ± 0.25^{Aa}	12.59 ± 0.25^{Aa}
Josephine (30)	43.44 ± 0.35^{Aa}	50.61 ± 0.94^{Bb}	51.98 ± 0.49^{Bbc}	8.05 ± 0.17^{Aa}	8.01 ± 0.09^{Aa}	7.95 ± 0.30^{Bb}	12.47 ± 0.11^{Aa}	11.73 ± 0.42^{Ba}	11.64 ± 0.17^{Bb}
Josephine (50)	46.67 ± 0.81^{Ba}	52.60 ± 0.30^{Cb}	55.11 ± 0.28^{Cbc}	8.05 ± 0.04^{Aa}	7.31 ± 0.08^{Bb}	7.25 ± 0.12^{Cb}	12.31 ± 0.15^{Aa}	11.52 ± 0.07^{Bab}	10.87 ± 0.24^{Cb}
Josephine (100)	50.01 ± 0.73^{Ca}	54.99 ± 0.41^{Db}	58.35 ± 0.25^{Dc}	7.32 ± 0.08^{Ba}	6.75 ± 0.16^{Cb}	6.64 ± 0.07^{Dc}	12.05 ± 0.51^{Ba}	11.52 ± 0.31^{Bab}	10.33 ± 0.32^{CDb}

Values are expressed as mean ± standard deviation (n = 3). Mean with different superscript capital letters within column and superscript lower case letters within row are significantly different (P < 0.05).

Table 6. Total microbial count of macerated broiler breast meat at different concentrations and durations.

Concentration (%)	Microbiological load (total microbial count log CFU/g)		
	maceration duration (min)		
	15 min	25 min	35 min
Control (DW)	4.35 ± 0.03	4.35 ± 0.03	4.35 ± 0.03
Josephine (30)	4.34 ± 0.06	4.18 ± 0.02	4.10 ± 0.02
Josephine (50)	4.26 ± 0.04	4.16 ± 0.03	4.03 ± 0.05
Josephine (100)	4.07 ± 0.02	4.03 ± 0.06	3.98 ± 0.03

Values are expressed as mean ± standard deviation (n = 3).
Notes: 100%, 50%, and 30%, concentration of the core extract; DW, distilled water; 15, 25 and 35 min, maceration duration.

citrus extract was the primary factor that affected the reduction of microorganisms. According to Alvarado and Mckee (2017), most microorganisms slowed their growth in an acidic environment. This statement is also supported by Kotzekidou *et al*. (2008), which stated that pineapple extract could suppress the growth of Escherichia coli O157:H7 EDL-933. The pineapple extract contains active substances such as terpenoids and phenolic compounds, and these compounds attach to the bacterial membrane and deplete the metabolic energy of bacteria. Our study found that samples macerated in 100% concentration of the core extract for 35 min had the lowest microbial count (3.98 Log CFU/g).

Conclusions

The maceration technique using 100% concentration of the core extract (Josephine variety) for 35 min shows the most significant meat tenderisation effect compared to other concentrations and durations used in this study. The hardness and chewiness of the broiler breast meat were reduced. Pineapple core extract is applicable as a meat tenderiser in the food industry, which may increase the demand for local pineapple core.

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Author Contributions Statement

C.H.W. conducted the experiment, C.H.W. and N. H. analysed the findings, and C.H.W., N. H., and N. S.M. wrote and edited the manuscript.

Additional information

The authors declare no conflict of interest.

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