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AEROBIC PLATE COUNT, pH, AND SELECTED SENSORY PARAMETERS OF THAWED AND FRESH PORK AT A PUBLIC MARKET IN CALAMBA, LAGUNA, PHILIPPINES

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ABSTRACT

Philippine public market sells unrefrigerated imported frozen meat products that may pose a public health concern. This study assessed the quality of thawed and fresh pork using aerobic plate count (APC), pH and sensory parameters (color, odor and presence of slime). Fifteen samples for each type of pork were collected in a public market at Calamba, Laguna, and divided into testing schedules and minced. Serial dilution, pour plating, incubation and APC (cfu/ g) were done; pH was determined; color and odor were ranked; and presence of slime was noted. Significant findings include higher APC for fresh pork, remarkably at 5h; higher increase in pH, mean color rank and mean odor rank for thawed pork; and higher peak percent slime positive for thawed pork. These findings suggest that fresh pork sold in Philippine market settings has a better keeping quality than thawed pork, and that thawed pork showed earlier signs of spoilage.

Key words: biodeterioration, discoloration, fresh products, microbial flora, pork, thawing

INTRODUCTION

Frozen meat importation in the Philippines has been on the rise (Apolinares *et al*., 2011) and this resulted to selling of unrefrigerated imported frozen meat in public markets. Imported meat was originally intended for sale in restaurants and supermarkets where there are available freezers for storage. The Department of Agriculture's Administrative Order No. 6 required frozen imported meat to be kept at a temperature no higher than 0°C during its handling, storage, distribution and sale. Given the cold storage disruptions and the lack of refrigeration available in Philippine markets for frozen meat products, this presents a significant public health concern. Frozen meat products are typically chopped and displayed in ambient tropical temperature in public market stalls, transforming the frozen meat into thawed meat state.

It is, therefore, the goal of this study to provide preliminary data on some parameters that determine the quality of meat sold at Calamba public market. Parameters detecting meat spoilage such as aerobic plate count and some sensory parameters like color, odor and slime-formation, were determined for both thawed and fresh pork given the same environment conditions similar to the public market. The pH was also measured and associated to the keeping quality of the meat. Bacterial growth rate was associated with the spoilage rate, and comparison of results between thawed and fresh pork was determined.

MATERIALS AND METHODS

Two hundred fifty grams (250 g) each of thawed and fresh pork were collected

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from 30 different meat stalls in Calamba wet market, making a total of 30 meat samples (15 thawed; 15 fresh). Collection was done using non-probability purposive sampling. Based on the label displayed on the boxes containing the thawed pork, sources were identified to be from the countries: Belgium, Australia, Canada, Denmark, France, Spain and Holland. Samples were individually placed in sterile plastic containers, labeled and sealed. Packed thawed meats were placed in a Styrofoam container to preserve the initial temperature of meat. Packed fresh meats were placed in a market bag. These samples were then brought to the laboratory, which took around 30 minutes of travel period.

The samples were divided and distributed at different exposure times or according to testing schedules, which have an interval of 5 hours (h) (Bruckner, 2010): 0, 5, 10, 15, 20, 25, and 30 h. Twenty grams of pork, placed in a sterile Petri dish without cover, was left exposed at room temperature at its designated exposure time. Doing this simulated that of the environmental temperature in the market. After exposure, 5 g of pork was weighed (SPO 51, Scaltec Instruments, Heiligenstadt Germany) and minced. This was transferred to a beaker with 495 ml sterile distilled water and was mixed to make a 1:100 dilution. One ml aliquot was transferred to a test tube containing 9 ml sterile distilled water to make a 1:1000 dilution. Same procedure was done to make a dilution of 1:10,000 and 1:100,000. One ml from each dilution was poured in a Petri dish, replicated once, and added with 15 ml plate count agar. It was allowed to solidify and then incubated at 35°C for 24 to 48 hours (Datta *et al.*, 2012). After incubation, colony forming units (cfu)/ gram were counted for each plate. The counts were averaged for the replicates. The final count was obtained by taking the average of the counts per dilution to get the mean aerobic plate count (APC).

Bacterial growth rate was computed by subtracting the initial mean APC at 0 h from the final at 30 h, divided by the total hours of exposure (30 h). Results were compared to determine the pork type that has a faster bacterial growth rate.

The pH of meat was taken by dipping the pH pen (pH600, Milwaukee Instruments, USA) (calibrated to pH 7.0 using phosphate buffer solution) to the homogenate which was made of 10 ml sterile distilled water and 10 g minced pork from each testing schedule. Results were correlated to APC.

Sensory evaluation of the meat was conducted at every testing schedule. The attributes evaluated included color, odor and presence of slime. Color and odor were ranked in a scale of 1 to 6, based on intensity (Remm *et al.*, 2011). Color was ranked from 1 (light pinkish brown) to 6 (dark reddish brown). Odor was ranked from 1 (hardly noticeable) to 6 (very strong). Slime formation was observed if present or absent (Bruckner, 2010). Results were correlated to APC.

Mean values at every exposure time was determined for parameters such as APC, pH, color ranking and odor ranking for each type of pork. Percentage of meat samples positive with slime was computed. To compare the APC, pH, color ranking and odor ranking between thawed and fresh pork, results per parameter were plotted in a line graph. For percent slime positive, results were shown using a bar graph. For every parameter, significant difference between thawed and fresh pork was determined using Mann-Whitney U Test established at $p < 0.05$. Association between APC and pH, color and odor was determined using Spearman's Correlation Coefficient. Strength of association between variables is based on qualitative interpretation of the correlation coefficient: $r \leq |0.2|$ = very weak, $|0.2| < r < |0.4|$ = weak, $|0.4| < r < |0.6|$ = moderate, $|0.6| < r < |0.8|$ = strong and $r > |0.9|$ = very strong.

The shelf life of pork was determined based on the time when bacterial count has reached 10^7cfu/g or 7 log10 cfu/g and when presence of off-odor and slime formation has been observed. Presence of slime was associated to the cfu/ g level above 7 log_{10} cfu/g using Phi Coefficient. Degree of association between variables is based on qualitative interpretation of the correlation coefficient: $r \leq 0.10$ = weak, $0.1 < r < 0.3$ = moderate and $r > 0.3$ = strong. All data were analyzed using SAS Base Statistical Program (SAS Institute, USA).

RESULTS AND DISCUSSION

Bacterial growth in thawed and fresh pork is shown in Fig. 1, where the actual mean APC (cfu/ g) is plotted in a line graph. Aerobic plate count reflects the quality and shelf life of the meat. High APC means poor meat quality and reduced shelf life (Eisel *et al.*, 1997; Bogere and Baluka, 2014). Between thawed and fresh pork, a pronounced difference in APC was only observed at 5h exposure in Fig. 2, showing the logarithmic mean APC. At this particular time, APC of fresh pork was 1.21×10^7 cfu/g, which is about 12 times higher than the APC of thawed pork at 9.77×10^5 cfu/g. But as exposure time increases, the APC of both types of pork no longer have significant differences ($p > 0.05$). The possible reason for this is that the thawed pork has an initial meat temperature lower than that of the fresh pork. And the lower the temperature, the longer the lag phase of bacterial growth (Cegielska-Radziejewska *et al.*, 2008). Hence at 5h, the lag phase of bacterial growth of thawed pork was simply prolonged. However, as it was being continually exposed to ambient temperature, APC dramatically increased to about 15-fold, from 9.77×10^5 to

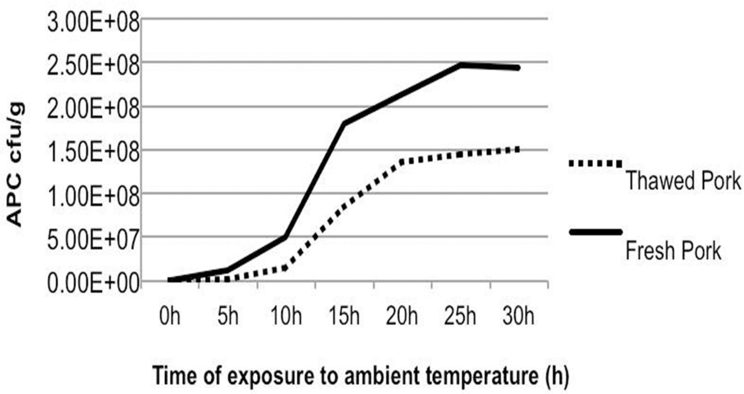

Fig. 1. Mean APC (cfu/g), taken at different testing schedules at ambient temperature, showing bacterial growth of thawed pork and fresh pork at Calamba public market.

1.43×10^7 cfu/g at 10h, as compared to the 4-fold APC increase in fresh pork from 1.21×10^7 to 4.96×10^7 cfu/g. This can be attributed to the effects of freezing and thawing to the chemical composition of meat. When the pork was exposed at a considerable period of time at ambient temperature, conditions were no longer inhibitory to the growth of bacteria. And since freezing and thawing effects on the pork has resulted in exudate formation, increasing the moisture and bringing out the nutrients, this has made the conditions very favorable for the bacteria to resume to its original activity (Leygonie *et al.*, 2012).

APC higher than 10^7 cfu/ g suggests a poor hygienic quality and makes the meat

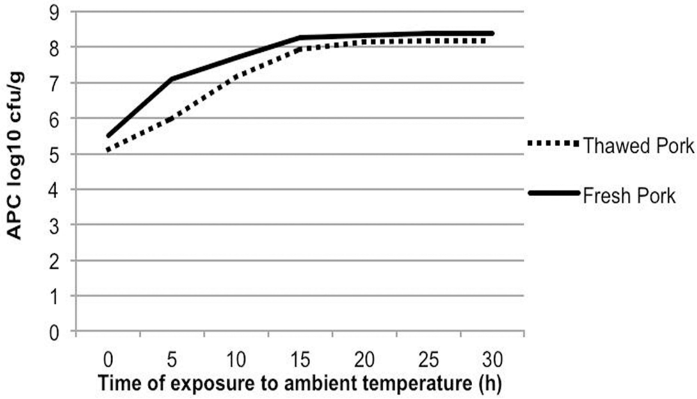

Fig. 2. Logarithmic mean APC (log cfu/g), taken at different testing schedules at ambient temperature, of thawed and fresh pork at Calamba public market.
[1] There is a significant difference (p = 0.0021) between APC of thawed and fresh pork.

unsuitable for consumption (Cohen *et al*, 2006). APC of thawed meat reached the 10^7 cfu/ g level at 10h, while fresh pork reached it at 5h. Moreover, bacterial growth rate showed that fresh pork has a faster change in mean APC from initial to the final exposure time, having the rate of 8.16 x 10^6 cfu/g/h, while thawed pork has the rate 5.03 x 10^6 cfu/g/h. Using the Mann-Whitney U Test (see *Table 1*), significant difference between the APC of the two types of pork was determined. At 5h exposure time, the APC of fresh pork was significantly higher ($p = 0.0021$) at 7.08 $\log_{10}$ cfu/g (1.21 x10^7 cfu/g) than the APC of thawed pork at 5.99 $\log_{10}$ cfu/g (9.77 x 10^5 cfu/g). APC on other testing schedules, i.e. on

Table 1. Statistical analysis showing parameters, taken at different testing schedules at ambient temperature, with statistical significance comparing thawed pork at Calamba public market.

TS	Thawed Pork			Fresh Pork		
	Mean APC (cfu/g)	**Mean pH (r)**	**Mean Odor Rank (r)**	**Mean APC (cfu/g)**	**Mean pH (r)**	**Mean Odor Rank (r)**
0h	1.29×10^5	6.16 (-0.2466)	1 ([1])	3.13×10^5	6.13 (0.1961)	1 ([1])
5h	9.77×10^{5a}	6.1 (0.4145)	1 ([1])	1.21×10^{7a}	6.09 (0.3478)	1.07 (-0.3858)
10h	1.43×10^7	6.08 (0.3504)	2 (0.5261[2])	4.96×10^7	6.06 (0.1765)	2 (0.1752)
15h	8.51×10^7	6.37 (0.2383)	3.13 (0.2835)	1.80×10^8	6.19 (0.7368[2])	3.2 (0.1494)
20h	1.36×10^8	6.62 (0.4314)	4.4[b] (0.5064)	2.13×10^8	6.33 (0.4721)	3.47[b] (0.7591[2])
25h	1.44×10^8	6.47 (0.2706)	5.2[c] (0.6728[2])	2.48×10^8	6.33 (0.2361)	4.27[c] (-0.0845)
30h	1.51×10^8	6.75 (0.1299)	4.93 (-0.1163)	2.45×10^8	6.51 (-0.1590)	5.13 (0.1737)

0, 10, 15, 20, 25 and 30h, yielded no significant differences ($p > 0.05$).

The normal muscle pH of a live animal is about 6.5-6.8, and in some cases, 7.2. At post-mortem, this declines to 5.4 to 5.8 due to the degradation of glycogen which yields a final product of lactic acid. Lactic acid accumulates hence the drop in pH (Gill and Newton, 1978; Lawrie, 1998; Bruckner, 2010). Thus, the pH of fresh pork follows the normal pattern of pH level changes at 5 and 10h (Table 2). For the thawed pork, pH should be lower than prior to freezing, which is expected to be at the ultimate pH level of 5.4 to 5.8 (Leygonie *et al.*, 2011). The mean pH of thawed pork at 0h is higher than expected. In the reports made by Ruiz-Capillas and Moral (2001) and Masniyom *et al.* (2002), an increase in pH during storage period can be due to the production of basic compounds such as ammonia and trimethylamine as well as other biogenic amines. The shift in pH trend, from decreasing to increasing, for both types of pork was evident at 10h and beyond. This is due to proteolysis in spoiling meat (Agunbiade *et al.*, 2010), which raises the level of amines that causes the continuous increase in pH. The implication of high pH is that it would provide an environment no longer inhibitory to the growth of bacteria. However, no correlation existed between APC and pH, as also reported in the study of Bruckner

Table 2. Change in mean pH, taken at different testing schedules at ambient temperature, of thawed and fresh pork at Calamba public market.

Testing Schedule	Mean pH	
	Thawed Pork	Fresh Pork
0h	6.16	6.13
5h	6.10	6.09
10 h	6.08	6.06
1 h	6.37	6.19
20 h	6.62	6.33
25 h	6.47	6.33
30 h	6.75	6.51

(2010). Overall, there was a more rapid increase in pH for the thawed pork.

Using Mann-Whitney U Test (see *Table 1*), no significant difference was observed between the pH of the two types of pork. To check for correlation of APC with this parameter, Spearman's Correlation Coefficient was used. The pH of fresh pork at 15h revealed a strong association with APC (if $\alpha=0.05$).

Table 3 shows the mean color rank of thawed and fresh pork at different testing schedules. Color is an important factor that influences the preference of customer because many consumers use meat color as an indicator of freshness and wholesomeness (Limbo *et al.*, 2010). As the exposure time increases, it was observed that the color of the meat changed from light pinkish brown to darker shade of reddish brown. This increase in hue is due to the gradual oxidation of myoglobin and accumulation of metmyoglobin with time (Mancini and Hunt, 2005; Ruiz de Huidobro *et al.*, 2003). The color of thawed pork in contrast with fresh pork was lighter at 0h but darker at 30h. Statistically, there is no significant difference in the color of the two types of pork, however, based on the data, change in meat color is much faster in thawed meat. Color is not associated with APC but is associated to pH, as mentioned in a study by Huff-Lonergan and Lonergan (2005). Pork with higher pH is darker in color while that with lower pH is lighter in color.

Table 3. Mean color rank, taken at different testing schedules at ambient temperature, of thawed and fresh pork at Calamba public market.

Testing Schedule	Mean Color Rank	
	Thawed Pork	Fresh Pork
0h	2.67	3.07
5h	3.00	3.07
10 h	3.40	3.53
1 h	3.80	3.60
20 h	4.40	4.00
25 h	4.20	3.93
30 h	4.87	4.27

Using Mann-Whitney U Test (see *Table 1*), no significant difference was observed between the colors of the two types of pork. Spearman's Correlation Coefficient revealed no association between APC and mean color rank.

Thawed pork has a statistically significant stronger putrid odor as compared to fresh pork, especially at 20 and 25h (Table 3). This may have been due to the effects of freezing. During freezing, there is denaturation of proteins. This results in exudate formation at thawing, which then leads to increase in moisture. The moisture lost during thawing is rich in proteins, vitamins and minerals derived from the structural disorder caused by the freezing process, which consequently provides an excellent medium for microbial growth (Leygonie *et al.*, 2012). Microbial loads from 10^7 cfu/g have been associated with off-odors and when the loads increase to as high as 109 cfu/g the meat becomes putrid (Dainty *et al.*, 1985; Jay, 2000). It has also been reported that metabolization of meat's low molecular substances, such as sugar and free amino acids and the release of undesirable volatile metabolites contribute to the development of organoleptic spoilage. As soon as the glucose present in aqueous phase has been exhausted other substrates are consequently utilized by metabolizing microorganisms to produce odoriferous nitrogenous compounds, the most predominant of which is ammonia (Pearson and Muslemuddin, 1968).

Using Mann-Whitney U Test (see *Table 4*), there is a significant difference between the mean odor ranks of thawed and fresh pork at 20h ($p = 0.0089$) and 25h ($p = 0.0146$). Spearman's Correlation Coefficient showed a moderate association ($p = 0.044$) between mean odor rank and APC of thawed pork at 10h, and a strong association ($p = 0.006$) at 20h. There is also a strong association ($p = 0.001$) in fresh pork at 20h.

Generally, a higher percentage of slime formation was observed in thawed pork. The probable reason for this is that freezing and thawing results in formation of exudate. And it was reported that there is a correlation between thawing rate and exudate formation (Leygonie *et al.*, 2012). Añón, and Cavelo (1980) concluded that a decrease in thawing time to below 50 minutes resulted in a decrease in exudate. This was attributed to the melting of ice in the extracellular spaces causing an increase in water activity, resulting in the net flow of water into the intracellular spaces and its subsequent reabsorption by the dehydrated fibers. These authors suggested that at increased rates of thawing, the rate at which water becomes available exceeds the rate at which the fibers can reabsorb water, with the excess water being excreted as exudate. It was also proposed that an increased rate of thawing caused less exudate to form. In the study, it can be inferred that thawing rate was prolonged considering the length of time the cold chain was disrupted, i.e. in

Table 4. Mean odor rank, taken at different schedules at ambient temperature, of thawed and fresh pork at Calamba public market.

Testing Schedule	Mean Odor Rank	
	Thawed Pork	Fresh Pork
0h	1.00	1.00
5h	1.00	1.07
10 h	2.00	2.00
1 h	3.13	3.20
20 h	4.40[a]	3.47[a]
25 h	5.20[b]	4.27[b]
30 h	4.93	5.13

[a] Means having the same superscript within a row are significantly different from each other (p = 0.0089)
[b] Means having the same superscript within a row are significantly different from each other (p = 0.0146)

transportation and in display of pork in meat stalls, hence the high yield of slime formation in thawed pork.

Presence of slime can be associated with an APC of 10^7 cfu/g or 7 log_{10} cfu/g. By doing the Phi Coefficient statistical analysis, it was revealed that presence of slime is indeed strongly associated ($p = <0.0001$) to having APC greater than 10^7 cfu/g or 7 log_{10} cfu/g at $\alpha=0.05$ for any type of pork.

CONCLUSION

The study determined meat quality parameters of fresh and thawed meat i.e. aerobic plate count, pH, color, odor, and presence of slime, at a public market in Calamba. Although the APC is higher in fresh pork, findings reveal that fresh pork has a better keeping quality as compared to thawed pork, taking into consideration the observations in pH, color, odor and slime. Taking into account the Filipino consumers' preference for fresh meat typically marketed in unrefrigerated displays and the surge of imported frozen meat being sold side by side with fresh meat, this study demonstrates the need to study the dynamics of meat spoilage in the Philippine market scenario.

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