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Effect of different packaging conditions and temperatures on physicochemical and microbiological quality of Tualang honey-marinated chicken meat

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ABSTRACT

The present study evaluated the microbial quality, pH, and water activity of Tualang honey (TH) marinated chicken breast meat under various temperatures and packaging conditions. Chicken breast meat samples were marinated in marinades containing 50% TH for 24 h at 4 °C. The samples were drained and packaged under aerobic and vacuum packaging conditions and stored at 5, 10, and 25 °C for 7, 4, and 2 days, respectively. The highest increase in TPC was noted in samples stored at 25 °C and the lowest in samples stored at 5 °C. At 10 °C until 12 h storage, the total plate count was 4.84 log CFU/g, and for 5 °C at 72 h, the total plate count was 5.82 log CFU/g. The pH value at 25 °C differed significantly ($p<0.05$) among the control, honey-marinated chicken with normal packaging (HCNP), and honey-marinated chicken with vacuum packaging (HCVP) samples. Control samples had the highest pH and water activity as compared to other samples at all temperatures studied. Thus, marinating chicken breast meat with 50% Tualang honey marinades improved the microbial quality, and this effect was better observed at low temperatures and under vacuum packaging.

***Key words*:** Chicken meat, marination, Tualang honey, microbial quality

INTRODUCTION

Spoilage of meat due to microbial growth and oxidative changes remains a major challenge for the meat sector. About 23% of the total meat produced is wasted due to spoilage throughout the food chain (Lipinski, 2020). The maximum food wastage occurs at the consumption level (60%), followed by manufacturing (20%), distribution (12%), primary production, and post-harvest (3.5%) (Karwowska *et al*., 2021). During storage, lipid oxidation,

and microbial growth cause the deterioration of the quality attributes of the product. Lipid oxidation is responsible for the reduction in nutritional quality as well as changes in flavor, whereas microbial contamination can cause public health hazards and economic loss owing to food poisoning and meat spoilage. Thus, for maintaining meat quality, the application of suitable agents having both antimicrobial and antioxidant activities is useful for extending shelf life and preventing economic loss (Umaraw *et al*., 2019). The application of natural preservatives as an alternative to synthetic preservatives in meat processing is gaining consumer acceptance due to the adverse effect of synthetic compounds on consumer health, issue of residual levels, and higher preference for natural foods (Awad *et al*., 2021, 2022; Kumar *et al*., 2020).

The traditional preservation methods that were practiced in ancient times include salt, sugar, spices, and wood smoke. Nowadays, with the development of new technology, more advanced methods of preservation have come to light, such as chemical antimicrobial agents and many organic acids, in order to achieve a longer shelf and enhanced protection from microbial spoilage. The presence of chemical residues in food, which has become a growing concern, has led to an increased demand for nontoxic natural preservatives. Marinating meat with suitable marinades having natural preservatives not only improves the quality of meat but also its shelf-life (Alvarado and McKee, 2007; Kumar *et al*., 2023; Roslan *et al*., 2019). Packaging has an important role in preserving the quality of meat and meat products by controlling exposure to oxygen, and light and limiting access to microbes (Umaraw *et al*., 2018, 2020).

Tualang honey (TH) is a multi-floral honey produced by rock bees or giant honeybees (*Apis dorsata*). It is a rich source of various bioactive compounds exerting antioxidant and antimicrobial activities, such as flavonoids (kaempferol, apigenin, naringenin, luteolin, and catechin) and phenolic acids (cinnamic, coumaric, syringic, benzoic, gallic, and caffeic acids) and 5-hydroxymethyl furfural (Chew *et al*., 2018; Kishore *et al*., 2011). The phenolic content in Malaysian TH was reported at 83.96 ± 4.53 mg GAE/100 g and antioxidant capacity at 53.06 ± 0.41 mg AAE/ g (Kishore *et al*., 2011). In addition to antioxidant and antibacterial effects, it also exerts anti-inflammatory, anticarcinogenic, therapeutic, and wound-healing, and protects against neurodegeneration (Ahmed and Othman, 2013; Azman *et al*., 2021; Kishore *et al*., 2011; Tan *et al*., 2009).

Due to its antioxidant and antimicrobial effects, TH could be a potential agent for extending the shelf-life and quality of meat products. Limited research exists on the application of TH in marination to improve the quality and shelf-life of marinated meat products. Thus, the present study was undertaken to evaluate the effect of TH-marinated chicken in different packaging conditions and at different temperatures on microbiological quality, pH, and water activity.

MATERIALS AND METHODS

Experimental design: Tualang honey (TH) was purchased from a hypermarket in Seri Kembangan, Selangor, Malaysia. The pH of Tualang honey used in this study is pH 3.72. A marinade solution containing 50% Tualang honey in filtered water was prepared. The chicken breast meat was obtained from a hypermarket in Seri Kembangan, Selangor, Malaysia, and transported to the Laboratory of Food Microbiology under chilled conditions in ice boxes. The skin and fat of the chicken breast were immediately removed.

The chicken breast meat was marinated by adding 50% Tualang honey marinades at a 1:2 ratio in sterile polypropylene bags. The bags were hermetically sealed and gently agitated by hand to ensure even distribution of marinades. The samples were marinated by keeping them at 4 °C for 24 h. After 24 h, the excess liquid was allowed to drain off for 5 min at room temperature. Control samples were stored without marination. The control and marinated samples were aerobically and vacuum packaged (QUIWARE® Pro VS188, Malaysia) in polypropylene bags, and stored under three time-temperature combinations, viz., refrigeration at 5 °C for 168 h (7 days), chilling at 10 °C for 96 h (4 days) and at ambient temperature 25 °C for 48 h (2 days). For each control and treatment, the chicken samples were packed individually packaged. The samples were regularly assessed for total plate count (TPC), pH, and water activity at every 24 h and 12 h interval, respectively.

Microbiological analysis: The microbiological quality of honey-marinated chicken samples, as well as control samples without marination, were tested in terms of total plate count (TPC) by following the methods as described by the American Public Health Association (APHA, 2001). Triplicate plates were prepared and microbial counts were expressed as colony forming units per gram (CFU/g). Sample preparation and serial dilution performed under aseptic conditions, near flame in pre-sterilized horizontal laminar flow apparatus (ESCO LifeSciences Group, labculture, Class II, Pennsylvania, USA).

A 10 g of the minced chicken breast sample was homogenized in a stomacher (Stomacher Lab-Blender 400) with 90 ml of 0.1% peptone water. Serial dilution was prepared up to dilution factor 10^{-5}. A 0.1 mL of each serial dilution was pipetted into the plate count agar for measuring total plate count (TPC), spread uniformly by using a

sterile spreader. The plates were incubated at 35°C for 48 h, and all single colonies on the agar were recorded and calculated.

Determination of pH: The pH of meat samples was measured at 28 ± 2°C using pH meters (Mettler-Toledo, Switzerland). The pH of the homogenized chicken meat samples was determined in a 10% aqueous honey solution using a digital pH meter after it was calibrated at pH 4.0, 7.0, and 9.0 using standard buffer solutions (Nor-Khaizura et al., 2019). After homogenizing and plating, the pH of the chicken sample was taken every 12 h using a pH meter.

Determination of water activity: The water activity of homogenized chicken samples was determined by using an electronic dew-point water activity meter Aqualab Series 3 model TE (United State of America) equipped with a temperature-controlled system which allow to have a temperature stable sampling environment. After every 24 h, water activity was taken of the chicken sample.

Statistical analyses: All experiments were performed in duplicate, and the results were expressed as mean values with standard deviations (SD). Significant differences at different time durations among control, aerobic, and vacuum-packaged samples were obtained by One-Way Analysis of Variance (ANOVA, Tukey's Multiple Range Test). The level of significance was considered 5%.

RESULT AND DISCUSSION

Microbiological quality (total plate count)

A significant difference ($p<0.05$) between the samples in terms of storage time and treatment was recorded. At 5 °C at 0 h, the initial TPC for control was 5.80 log CFU/g, and for honey-marinated chicken with normal packaging (HCNP) and honey-marinated chicken with vacuum packaging (HCVP), it was recorded at 4.72 log CFU/g (Fig 1). At 12 h, HCNP and HCVP showed a significant decrease ($p<0.05$) in TPC count as compared to control, which noted an increasing trend. The control samples showed a gradually increasing trend until the 7 days.

The total plate count (TPC) at 10 °C, at 0 h, there was no significant difference ($p>0.05$) between the control, HCNP and HCVP (Fig 2). After 12 h storage, the TPC of control and HCNP samples were comparable and significantly ($p<0.05$) higher than HCVP samples. However, after 24 storages onwards, the highest TPC was recorded for control samples and significantly higher ($p<0.05$) than HCNP samples, which in turn recorded significantly higher TPC values than HCVP samples (C>HCNP>HCVP). At 24 h, the highest TPC was recorded for control samples (7.02 log CFU/g), followed by that of HCNP (6.02 log CFU/g) and the lowest for HCVP (5.75 log CFU/g). After 24 h, the TPC of control samples was higher ($p<0.05$) than both HCNP and HCVP.

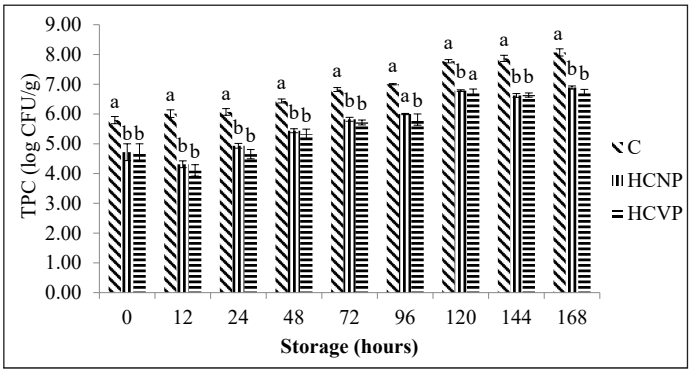

Fig. 1. Total plate count of unmarinated chicken (control) and honey-marinated chicken with normal (HCNP) and vacuum packaging (HCVP) at 5 °C, different superscript (a, b--) represent significant ($p < 0.05$) difference between the treatment

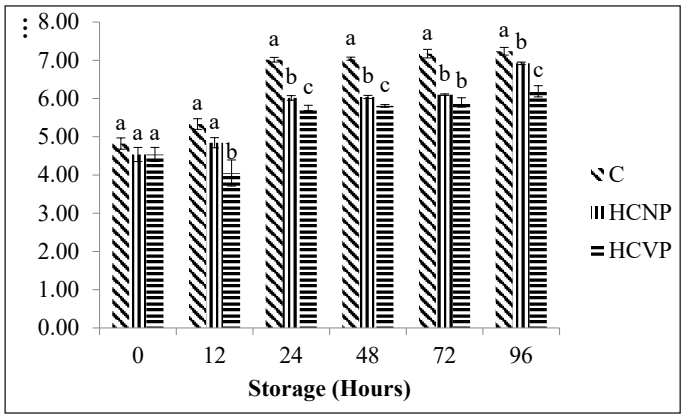

Fig. 2. Total plate count (TPC) of unmarinated chicken (control) and honey-marinated chicken with normal (HCNP) and vacuum packaging (HCVP) at 10 °C, different superscripts a, b, c denotes significant ($p < 0.05$) difference between the treatment

At 25 °C, the TPC of control samples was significantly higher ($p<0.05$) than marinated chicken samples in aerobic and vacuum packaging conditions (Fig 3). The TPC of HCNP and HCVP samples were recorded as comparable at 0 h, and afterward, a significantly ($p<0.05$) higher TPC was recorded for HCNP samples than HCVP samples. After 24 h, the TPC of control, HCNP, and HCVP samples were observed to increase with 7.12, 6.12, and 5.39 log CFU/g, respectively.

Total plate count (TPC) is the common method used to indicate the microbiological quality of food. It gives a quantitative idea about the presence of microorganisms in food, such as bacteria, yeast, and molds in a sample. The control showed a higher count (up to 8 log CFU/g) at the end of storage, which is above the maximum limit (not more than 6 log CFU/g) (Stannard, 1997).

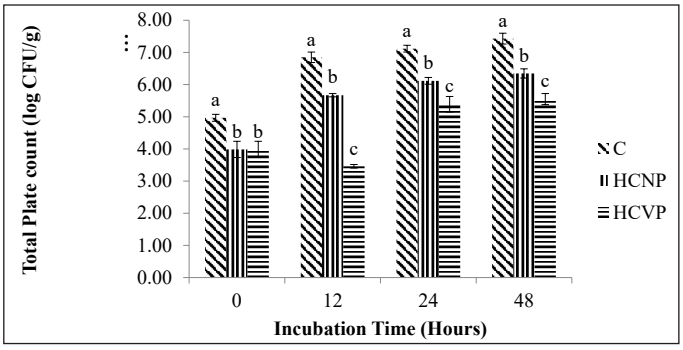

Fig. 3. Total plate count of unmarinated chicken (control) and honey marinated chicken with normal (HCNP) and vacuum packaging (HCVP) at 25°C. a, b, c significant ($p < 0.05$) difference between the treatment

The highest increase in TPC was noted in samples stored at 25 °C and the lowest in samples stored at 5 °C. On freshly processed poultry, mesophilic bacteria are found with an optimum growth temperature of about 35 °C. In contrast, the populations of bacteria that are found on spoiled poultry are psychrotrophic (readily multiply at a temperature between 20 to 30 °C and can also grow at refrigeration temperature). While at 10 °C until 12 h storage, the total plate count was 4.84 log CFU/g, which was satisfactory, and for 5°C at 72 h, the total plate count was within acceptable limits (5.82 log CFU/g). For the TH-marinated chicken with vacuum packaging (HCVP) at 25 °C, the total plate count was 5.55 log CFU/g after 48 h of storage.

The lower TPC count in the TH-marinated samples could be attributed to the antibacterial effect of TH due to the presence of phenolics, flavonoids, and furfurals in addition to the lower water activity of samples upon treating with honey (Yücel *et al*., 2005). The antimicrobial activity of Malaysian TH was also reported to have strong antibacterial activity against enteric and wound microorganisms (Tan *et al*., 2009). Similarly, a decreased microbial count in chicken breast treated with 20-30% honey stored for 7 days under vacuum conditions at 4 °C without adversely affecting meat quality attributes, including organoleptic attributes, was reported (Yücel *et al*., 2005). Similarly, the addition of 20% honey in chicken meat slices was observed to reduce TPC and coliform counts during 14 days of storage at 4 °C (El-Kalyoubi *et al*., 2014).

PH

The pH values of marinated chicken samples were recorded as acidic in nature, attributed to the acidic nature of the honey. The pH value at 25 °C differed significantly ($p<0.05$) among the control, HCNP, and HCVP (Table-1). Control had the highest pH as compared to other samples. After 12h, pH started to increase in all samples, but a rapid increase was recorded in the control samples, while HCNP and HCVP exhibited a gradual increase in pH values. This was due to the favorable temperature as food spoilage microorganisms started to multiply.

At 10 °C after 12 h, pH started to increase in all samples, and at the end of the control, HCNP and HCVP had a pH of 6.60, 5.52, and 5.34. The pH values started to increase gradually in all samples even in the control. At the end of incubation, the pH of the control, HCNP, and HCVP reached 6.19, 5.43, and 5.34. The highest pH was seen by the control sample, and the lowest pH was seen by HCVP in all temperatures and storage hours. There was a significant difference ($p<0.05$) between the samples in terms of storage time and treatment.

The pH of the meat has a special importance in its processing, directly influencing shelf life, color and quality of the meat. The pH range of pure honey varies between 3.2 to 4.5 *(Dan et al*., 2018), and the growth of many foodborne pathogens and spoilage microorganisms is optimal in the range of 4.2 to 7.4, which is higher than that of honey. The change in the pH of the honey-treated meat varies with the level of honey added to the marinade solution (Adeyanju and Ishola, 2014). The higher pH values of chicken meat samples were recorded with the increasing temperature of storage. Overall control samples recorded a higher pH value as compared to the HCNP and HCVP samples. This could be attributed to the antimicrobial effect of the honey (Chew *et al*., 2018).

Further vacuum packaging observed to have lower pH values, could be attributed to the dissociation of organic acids, lactic and acetic acid, accumulated in muscle tissue. Microbial growth inhibition of marinated chicken samples during storage can be due to the change that occurs in the chicken environment, which is caused by the marinade solutions, as all marinated samples demonstrated a significant reduction in the pH in comparison to the control samples. However, the inhibition could be attributed to the action of the flavonoids and phenolic compounds present in these marinades which are reported to have inhibitory effects on microbial growth (Istrati *et al*., 2015).

Water activity (a_w)

The microbiological safety of food is directly influenced by water activity. At 0 h, the water activity of all samples stored under different temperatures was observed to be comparable. Upon increasing the storage duration, the water activity of the TH-marinated meat samples was recorded as a

Table-1: pH of honey-marinated chicken breast meat stored under different temperatures and packaging conditions

Storage temperature	Sampling time (h)	Control	HCNP	HCVP
5 °C	0	5.88±0.01^{b}	4.85±0.01^{a}	4.85±0.01^{a}
	12	5.88±0.01^{b}	4.92±0.03^{a}	4.83±0.01^{a}
	24	5.92±0.02^{b}	5.08±0.01^{a}	5.01±0.02^{a}
	48	5.97±0.01^{c}	5.20±0.01^{b}	5.12±0.01^{a}
	72	5.97±0.01^{b}	5.20±0.02^{b}	5.12±0.01^{a}
	96	5.96±0.01^{c}	5.26±0.01^{b}	5.15±0.01^{a}
	120	6.02±0.01^{b}	5.30±0.03^{a}	5.21±0.01^{a}
	144	6.12±0.01^{b}	5.32±0.02^{a}	5.25±0.01^{a}
	168	6.19±0.03^{b}	5.43±0.04^{a}	5.34±0.02^{a}
10 °C	0	5.89±0.01^{b}	4.84±0.01^{a}	4.84±0.03^{a}
	12	5.93±0.02^{b}	4.93±0.01^{a}	4.90±0.01^{a}
	24	6.02±0.03^{b}	5.09±0.01^{a}	5.06±0.01^{a}
	48	6.11±0.03^{b}	5.23±0.01^{a}	5.13±0.03^{a}
	72	6.48±0.01^{b}	5.33±0.03^{a}	5.28±0.02^{a}
	96	6.66±0.01^{b}	5.22±0.01^{a}	5.34±0.01^{a}
25 °C	0	6.08 ± 0.04^{b}	4.87±0.01^{a}	4.87±0.01^{a}
	12	6.17±0.03^{b}	4.96±0.01^{a}	4.95±0.01^{a}
	24	6.40±0.03^{b}	5.28±0.01^{a}	5.13±0.02^{a}
	48	6.90±0.01^{b}	5.34±0.02^{a}	5.19±0.01^{a}

Values are Mean±SD, different superscript (a, b, and c) represent the significant difference between the samples, Control-sample without marination, HCNP- honey-marinated chicken with normal packaging, HCVP- honey-marinated chicken with vacuum packaging level of significance ($p<0.05$), n=6

decreasing trend. This could be due to the incorporation of TH, which has a low water activity (0.53). The highest water activity was seen by the control sample during the storage, while in both HCNP and HCVP, the water activities decreased until the end of the incubation (Table-2). However, the changes in water activity values were not in distinctive order throughout the storage period, especially for HCNP and HCVP samples at both 10°C and 5°C. There is a significant difference ($p<0.05$) between the samples in terms of storage time and treatment.

At 25 °C, water activity was in the range of 0.985 to 0.997. After 24 h incubation, control and HCNP had higher water activity as compared to HCVP. Water activity started to increase after 24 h incubation. There was no significant difference ($p>0.05$) between the samples in terms of storage time and treatment. At 10 °C, the water activity ranged between 0.946 to 0.995. Control had higher water activity starting from 24 h until the end of the storage period. The HCVP and HCNP samples were recorded with the lowest water activity.

The water activity of honey depends upon its glucose content (Gleiter *et al*., 2006) and moisture content (Zamora *et al*., 2006). The sugars present in TH can bind to water and make it unavailable for microbes, thereby reducing the water activity of the TH-marinated chicken breast samples. This could also be attributed to the antibacterial activity of honey (Yücel *et al*., 2005). A decrease in the moisture content in goat meat with increasing levels of honey was also reported (Raziuddin *et al*., 2021). In the food industry, sugars and sugar-concentrated products such as sucrose, dextrose, lactose, maltodextrins, molasses, corn syrup, starches, and honey are usually used in dried meat processing for enhancing flavor, reducing the harshness of salts and lowering the water activity (Raziuddin *et al*., 2021).

Table 2: Water activity of honey-marinated chicken breast meat stored under different temperatures and packaging conditions

Storage temperature	Sampling time (h)	Control	HCNP	HCVP
5 °C	0	0.995±0.005	0.995±0.006	0.995±0.005
	24	0.991±0.004[b]	0.988±0.001[a]	0.975±0.005[a]
	48	0.992±0.004[b]	0.968±0.001[a]	0.977±0.006[b]
	72	0.995±0.002[b]	0.967±0.008[a]	0.964±0.005[a]
	96	0.994±0.001[a]	0.958±0.007[a]	0.967±0.003[a]
	120	0.996±0.002[b]	0.986±0.002[a]	0.953±0.002[a]
	144	0.997±0.003[b]	0.968±0.007[a]	0.965±0.005[a]
	168	0.995±0.002[a]	0.952±0.004[a]	0.968±0.004[a]
10 °C	0	0.995±0.002	0.995±0.002	0.995±0.001
	24	0.993±0.001[b]	0.952±0.004[a]	0.947±0.003[a]
	48	0.994±0.001[b]	0.958±0.001[a]	0.955±0.004[a]
	72	0.995±0.003[b]	0.965±0.001[a]	0.961±0.002[a]
	96	0.996±0.002[a]	0.973±0.001[a]	0.961±0.002[a]
25 °C	0	0.994±0.006	0.994±0.007	0.994±0.006
	24	0.992±0.007	0.991±0.003	0.985±0.007
	48	0.997±0.003	0.994±0.001	0.996±0.003

Values are Mean±SD, different superscript (a, b, and c) represent the significant difference between the samples, Control-sample without marination, HCNP- honey-marinated chicken with normal packaging, HCVP- honey-marinated chicken with vacuum packaging level of significance ($p<0.05$), n=6

CONCLUSION

Marination of chicken breast meat samples in marinades with 50% Tualang honey inhibited the microbial quality and lowered the pH, with higher inhibition at lower temperatures, with vacuum packaged samples reported the lowest value at the end of storage. The water activity of the chicken meat samples was affected only at 5 and 10 °C, and at 25 °C, it was recorded as comparable within marinated and non-marinated samples.

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