



## EFFECT OF ACIDIC MARINATION ON THE QUALITY CHARACTERISTICS OF SPENT HEN KOBEBBA DURING FROZEN STORAGE

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### ABSTRACT

Marinated spent hen meats were used in the preparation of kobeba. The chemical, physico-chemical, microbiological and sensory properties of kobeba were evaluated during storage at -18°C for 5 months. Kobeba treated with lime juice had higher crude protein (14.03%), crude fat (7.78%), total ash (3.18%), carbohydrates (8.69%), cooking loss (5.62%), water holding capacity (3.41 cm<sup>2</sup>/0.3g) and plasticity (2.30 cm<sup>2</sup>/0.3g) and lower moisture (66.41%) and pH (5.96) than kobeba treated with propionic acid and acetic acid. Lime juice, acetic acid and propionic acid marination reduced total volatile nitrogen values by 25.82, 20.53 and 11.20% and thiobarbituric acid values by 33.82, 25.37 and 18.38%, respectively. Lime juice followed by acetic acid was more effective in reducing total bacterial, psychrophilic bacteria and yeast and mold counts than propionic acid. Kobeba marinated with lime juice had higher rating scores (7.68-7.98) for all sensory properties than kobeba marinated with propionic acid and acetic acid. Total volatile nitrogen, thiobarbituric acid, pH, water holding capacity and cooking loss of kobeba were increased as storage period increased. However, plasticity had an opposite trend. Water and fat retention of kobeba were not affected by storage period. At the end of storage period, kobeba had rating scores described as like slightly (6.19-6.45) for all sensory properties.

Chicken meats are very popular food around the world. Their consumption increased over the last decades. The popularity of chicken meats may be due to low production cost, low fat content and high nutritional value (Chouliara et al 2007). At the end of egg laying cycle (usually at 85-100 weeks) chicken had tough muscle, due to formation of a high amount of heat stable collagen. Therefore these meats needed equate processing to improve their acceptability (Now-sad et al 2000 and Ilayabharathi et al 2012).

Marination is important process for meat rich in connective tissue (Burke and Monahan, 2003). Marination process usually conducted by soaking or injecting meat with a solution containing ingredients such as organic acids, vinegar, lemon juice, soy sauce, brine, essential oils, salts, tenderizers, herbs and spices (Pathania et al 2010). Three mechanisms describe how the impact of acid marination improve tenderness of the meat, the first swelling caused by the pH of the muscle fiber and / or connective tissue, the second, speed up the weakness of muscle protein structure increasingly and the third increasing solubilization of collagen by heating process (Aktas et al 2003). Marination process increases product yield, reduces water loss during cooking and improves the shelf life of meat (Alvarado and McKee, 2007 and Kargiotou et al 2011). In this study spent hen meat was marinated in three different acidic solutions (lime juice, 1% propionic acid and 1% acetic acid) with 5% soy sauce separately for 30 min at room temperature. Kobeba was prepared from marinated spent hen meats. The chemical,

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physicochemical, microbiological and sensory properties of kobeba were evaluated during storage at -18°C for 5 months.

## MATERIALS AND METHODS

### Materials

#### Chicken meat:

Two years old chicken females (spent laying hens) weighing 5-6 kg were purchased from the local market in Cairo, Egypt. Chicken was slaughtered, allowed to bleed for 5 minutes, scalded for 2 min at 60°C, plucked by hand, eviscerated, rinsed with tap water, skinned, deboned and finally trimmed of fat, as required for the processing.

#### Spices:

Nine different dried natural herbs (5% cardamom, 15% cinnamon, 3% nutmeg, 3% ginger, 5% laurel leaves, 35% cuibeb, 15% cloves, 5% thyme and 14% white pepper) were purchased from the local market in Cairo, Egypt. The spices were mixed together then ground to pass through a 60 mesh sieve and kept in tight jar.

#### Texturized soy and Bulgur:

Texturized soy was obtained from Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. It was rehydrated by water at a ratio of 1:2 (w/v) and minced twice through 3 mm plate. Bulgur was purchased from the local market in Cairo, Egypt. It was washed then soaked in water at a ratio of 1:2 (w/v) for 2 hours and minced twice through 3 mm plate.

#### Other ingredients:

Salt, fresh garlic, fresh onion, cumin, black pepper and sunflower oil were purchased from local market, Cairo, Egypt.

### Methods

#### Preparation of kobeba

Kobeba was prepared as described by **Cetinkaya et al (2012)**. The whole chicken were deskinning, trimmed from fat and deboned. Lean chicken meats were divided into 4 portions. The first portion was considered as control. The second

portion was marinated with lime juice (lime juice:water, 1:3, v/v) and 5% soy sauce for 30min at room temperature. The third portion was marinated with 1% propionic acid and 5% soy sauce for 30min at room temperature. The fourth portion was marinated with 1% acetic acid and 5% soy sauce for 30min at room temperature. Appropriate amounts of each portion were subjected to grinding at 0.4 cm plate. The outside part of kobeba was composed from 45% of each portion, 38.5% bulgur, 5% texturized soy, 4% ground onion, 2% sodium chloride, 2% cumin, 1.5% spices, 1.36% ground garlic, 0.6% black pepper and 0.04% phosphate. While the inside part of kobeba was composed from 77.9% of each portion (fried meat), 4.5% fried ground onion, 13.1% texturized soy, 2.5% spices and 2% sodium chloride. Each part was mixed by hand and subjected to final grinding at 0.4 cm plate. The two parts together of each portion (50g of outside part and 5g of inside part) were formed into oval shape. Kobebas were placed on foam plate, wrapped with polyethylene film and stored at -18°C for 5 months. Kobebas were taken at specific time intervals for analysis.

#### Proximate composition

**AOAC (2012)** methods were used to determine moisture, crude fat, crude protein and total ash contents. Total carbohydrates content were calculated by difference.

#### Total volatile nitrogen

Total volatile nitrogen (TVN) was determined as described by **Winton and Winton (1958)**.

#### Thiobarbituric acid

Thiobarbituric acid value (TBA) was determined according to the procedure described by **Kirk and Sawyer (1991)**. The TBA values were expressed as mg malonaldehyd/kg of dry sample.

#### Cooking characteristics

##### Cooking loss, fat retention and moisture retention

Cooking loss, fat retention and moisture retention were determined as described by **Alesson-Carbonell et al (2005)** as follows:

$$\% \text{cooking loss} = \frac{\text{Uncooked kobeba weight} - \text{Cooked kobeba weight}}{\text{Uncooked kobeba weight}} \times 100$$

$$\frac{\% \text{fat retention}}{\times 100} = \frac{\text{Cooked kobeba weight} \times \% \text{fat in cooked sample}}{\text{Uncooked kobeba weight} \times \% \text{fat in uncooked sample}}$$

$$\frac{\% \text{moisture retention}}{\times 100} = \frac{\text{Cooked kobeba weight} \times \% \text{moisture in cooked sample}}{\text{Uncooked kobeba weight} \times \% \text{moisture in uncooked sample}}$$

### pH

The pH value was determined using 10% dispersion of sample in distilled water using a Jenway Digital pH meter (Model 3510).

### Water holding capacity and plasticity

Water holding capacity and plasticity were measured according to the filter-press method of Wierbicki and Deatherage (1958).

### Total bacterial count and psychrophilic bacteria

The procedure of APHA (1976) was followed for total bacterial count and psychrophilic bacteria count using the standard plate count agar and enumerated after incubation at 30°C for 3 days and 8°C for 5 days, respectively.

### Lipolytic bacteria count

Lipolytic bacteria were counted according to the methods mentioned by Difco manual (1984) using 10% olive oil. The Plates were incubated at 37°C for 5 days.

### Coliform bacteria count

The coliform bacteria were determined using MacConkey agar medium according to the method described by APHA (1976). The plates were incubated at 37°C for 24-48 hrs.

### Salmonella spp

The *Salmonella spp* was determined according to the method described by FAO (1979). The plates were incubated at 35°C for 24h.

### Yeast and mold counts

The procedures of Difco manual (1984) were followed for the determination of yeast and mold

counts using potato-dextrose agar medium. The plates were incubated at 20 – 25 °C for 5 days.

### Sensory evaluation

Kobeba treatments were fried in deep sunflower oil at 150°C for 5 min on each side. Sensory properties of kobeba treatments were carried out according to Mansour and Khalil (1999) by ten-trained panelists. Randomly coded samples were served to panellists individually. Five sensory attributes were evaluated (taste, odor, color, texture and overall acceptability) using nine-points hedonic scale for each trait where 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely.

### Statistical analysis

Statistical analysis was conducted according to Snedecor and Cochran (1994). A completely randomized 4 (marination type) × 6 (storage period) × 3 (replication) factorial designs was used. An analysis of variance was conducted using Costat version 6.311 (Copyright 1998-2005, CoHort software). When a significant main effect was detected, the means were separated with the Student Newman Keuls test. The predetermined acceptable level of probability was 5% ( $P \leq 0.05$ ) for all comparisons.

## RESULTS AND DISCUSSION

### Proximate composition of kobeba

Data in Table (1) showed that marinated kobeba had higher ( $p \leq 0.05$ ) crude protein, crude fat, total ash and carbohydrates contents and lower ( $p \leq 0.05$ ) moisture content than control kobeba. The increment in these components was attributed to the reduction in moisture content during marination process. Ergezer and Gokce (2011) found that acid marinated turkey breast meat had higher moisture and ash contents and lower protein and fat contents than control. Kobeba treated with lime juice had higher ( $p \leq 0.05$ ) crude protein, crude fat, total ash and carbohydrates contents and lower ( $p \leq 0.05$ ) moisture content than kobeba treated with propionic acid and acetic acid. There was no difference ( $P > 0.05$ ) in proximate composition between kobeba treated with propionic and acetic acid.

**Table 1.** Effect of marination type and storage at -18°C for 5 months on the proximate composition of kobeba

Treatment	Moisture (%)	Crude protein (%)	Crude fat (%)	Total ash (%)	Carbohydrates (%)
<b>Marination type</b>					
unmarinated	67.66 <sup>a</sup>	13.62 <sup>c</sup>	7.54 <sup>c</sup>	3.05 <sup>c</sup>	8.05 <sup>c</sup>
lime juice	66.41 <sup>c</sup>	14.03 <sup>a</sup>	7.78 <sup>a</sup>	3.18 <sup>a</sup>	8.69 <sup>a</sup>
propionic acid	66.93 <sup>b</sup>	13.95 <sup>b</sup>	7.66 <sup>b</sup>	3.11 <sup>b</sup>	8.51 <sup>b</sup>
acetic acid	66.78 <sup>b</sup>	13.85 <sup>b</sup>	7.66 <sup>b</sup>	3.09 <sup>b</sup>	8.56 <sup>b</sup>
LSD	0.32	0.11	0.10	0.04	0.11
<b>Storage period (months)</b>					
0	67.39 <sup>a</sup>	14.02 <sup>a</sup>	7.42 <sup>e</sup>	2.90 <sup>f</sup>	8.29 <sup>d</sup>
1	67.25 <sup>ab</sup>	13.96 <sup>ab</sup>	7.51 <sup>de</sup>	2.96 <sup>e</sup>	8.35 <sup>d</sup>
2	67.05 <sup>abc</sup>	13.91 <sup>ab</sup>	7.60 <sup>cd</sup>	3.07 <sup>d</sup>	8.42 <sup>cd</sup>
3	66.86 <sup>bc</sup>	13.85 <sup>bc</sup>	7.67 <sup>bc</sup>	3.16 <sup>c</sup>	8.50 <sup>bc</sup>
4	66.67 <sup>cd</sup>	13.76 <sup>cd</sup>	7.74 <sup>ab</sup>	3.24 <sup>b</sup>	8.59 <sup>ab</sup>
5	66.46 <sup>d</sup>	13.71 <sup>d</sup>	7.81 <sup>a</sup>	3.32 <sup>a</sup>	8.73 <sup>a</sup>
LSD	0.39	0.12	0.12	0.05	0.14

Means in the same column with different letters are significantly different ( $p \leq 0.05$ )

Moisture and crude protein contents of kobeba were significantly ( $p \leq 0.05$ ) decreased from the third month up to the end of storage. However, crude fat, total ash and carbohydrates of kobeba had an opposite trend. The reduction in moisture might be due to the drip loss and evaporation of moisture (Osheba, 2003 and El-Kordy, 2006). Rehab (2002) mentioned that the decrease in moisture content during the storage might be due to the reduction in protein solubility and subsequently the decrease in water holding capacity. However, the increment in crude fat, total ash and carbohydrates were attributed to the reduction in moisture and protein contents during frozen storage.

#### Total volatile nitrogen and thiobarbituric acid of kobeba

Data in Table (2) showed that marinated kobeba had lower ( $p \leq 0.05$ ) TVN values than control. The reduction in the TVN values was attributed to the effect of acidic marination as reported by Smaoui et al (2011) who found a reduction in the total volatile bases nitrogen contents in treated chicken thigh by lactic acid at different concentrations. Lime juice followed by acetic acid was more effective ( $p \leq 0.05$ ) in reducing TVN values than

propionic acid. Lime juice, acetic acid and propionic acid reduced TVN values by 25.82, 20.53 and 11.20%, respectively. The TVN values of kobeba were significantly ( $p \leq 0.05$ ) increased as storage period increased. The increase in TVN values might be due to the bacterial breakdown during storage of meat products (Osheba and Abd El-Bar, 2007). The increment in TVN values during storage period ranged between 22.06 to 204.70%.

Marinated kobeba had lower ( $p \leq 0.05$ ) TBA values than control. Lime juice followed by acetic acid was more effective ( $p \leq 0.05$ ) in reducing TBA values than propionic acid. This might be due to the strong antioxidant activity of bioactive compounds such as ascorbic acid, citric acid, phenolic compounds and flavonoids compounds present in lime juice (Vlahova-Vangelova and Dragoev, 2014). Lime juice, acetic acid and propionic acid reduced TBA values by 33.82, 25.37 and 18.38%, respectively. The TBA values of kobeba were significantly ( $p \leq 0.05$ ) increased as storage period increased. The increase in TBA values during storage period might be due to continuous oxidation of lipids and consequently the production of oxidative by-products. These results are in agreement with those obtained by (Khalafalla et al 2016) who observed a significant increase in TBA values with storage time in broiler chicken breasts meat. The

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**Table 2.** Effect of marination type and storage at -18°C for 5 months on the total volatile nitrogen and thiobarbituric acid of kobeba

Treatment	TVN (mg N/100g)	TBA (mg malonaldehyd/kg)
<b>Marination type</b>		
Unmarinated	12.86 <sup>a</sup>	0.544 <sup>a</sup>
lime juice	9.54 <sup>d</sup>	0.360 <sup>d</sup>
propionic acid	11.42 <sup>b</sup>	0.444 <sup>b</sup>
acetic acid	10.22 <sup>c</sup>	0.406 <sup>c</sup>
LSD	0.12	0.017
<b>Storage period (months)</b>		
0	5.53 <sup>f</sup>	0.283 <sup>e</sup>
1	6.75 <sup>e</sup>	0.302 <sup>e</sup>
2	9.43 <sup>d</sup>	0.352 <sup>d</sup>
3	12.37 <sup>c</sup>	0.429 <sup>c</sup>
4	15.14 <sup>b</sup>	0.568 <sup>b</sup>
5	16.85 <sup>a</sup>	0.699 <sup>a</sup>
LSD	0.15	0.020

Means in the same column with different letters are significantly different ( $p \leq 0.05$ )

increment in TBA values during storage period ranged between 6.71 to 146.99%.

The acceptability recommended rate for TVN and TBA in poultry meat products are 20 mg N/100g and 0.9 mg malonaldehyd/kg, respectively (**Egyptian standard specifications, 2009**). In the present study, TVN values (5.53 to 16.85 mg N/100g) and TBA values (0.283 – 0.699 mg malonaldehyd/kg) of kobeba were within the acceptable range.

**Water holding capacity, plasticity and pH value of kobeba**

Data in **Table (3)** showed that water holding capacity values of kobeba were increased ( $p \leq 0.05$ ) after marinating process. The lowest water holding capacity (highest value, 3.41  $\text{cm}^2/0.3\text{g}$ ) was recorded for lime juice kobeba. On the other hand, the highest water holding capacity (lowest value, 3.17  $\text{cm}^2/0.3\text{g}$ ) was recorded for control kobeba. The increment of water holding capacity values of kobeba by marination process was due to low pH which leading to reduce the ability to

bind water. These finding is in agreement with the results of **Alvarado and McKee (2007)** who found that the irreversible changes occur at pH below 4.5, after marination with weak organic acids or their salts, causing decrease in water holding capacity. Water holding capacity value of kobeba did not significantly ( $P > 0.05$ ) change until one month of storage. While, from the second month of storage gradual increases ( $P \leq 0.05$ ) in WHC values were observed until the end of storage period. The increment of WHC values of frozen kobeba during storage might be attributed to protein denaturation and loss of protein solubility (**Abd El-Qader, 2004 and El-Kordy, 2006**).

**Table 3.** Effect of marination type and storage at -18°C for 5 months on the water holding capacity, plasticity and pH value of kobeba

Treatment	WHC ( $\text{cm}^2/0.3\text{g}$ )	Plasticity ( $\text{cm}^2/0.3\text{g}$ )	pH
<b>Marination type</b>			
Unmarinated	3.17 <sup>c</sup>	1.85 <sup>d</sup>	6.30 <sup>c</sup>
lime juice	3.41 <sup>a</sup>	2.30 <sup>a</sup>	5.96 <sup>d</sup>
propionic acid	3.28 <sup>b</sup>	2.09 <sup>c</sup>	6.27 <sup>a</sup>
acetic acid	3.32 <sup>b</sup>	2.17 <sup>b</sup>	6.17 <sup>b</sup>
LSD	0.08	0.04	0.02
<b>Storage period (months)</b>			
0	2.81 <sup>e</sup>	2.22 <sup>a</sup>	5.84 <sup>f</sup>
1	2.86 <sup>e</sup>	2.19 <sup>a</sup>	5.97 <sup>e</sup>
2	3.16 <sup>d</sup>	2.13 <sup>b</sup>	6.11 <sup>d</sup>
3	3.44 <sup>c</sup>	2.09 <sup>c</sup>	6.23 <sup>c</sup>
4	3.67 <sup>b</sup>	2.02 <sup>d</sup>	6.38 <sup>b</sup>
5	3.87 <sup>a</sup>	1.97 <sup>e</sup>	6.51 <sup>a</sup>
LSD	0.11	0.03	0.02

Means in the same column with different letters are significantly different ( $p \leq 0.05$ )

Plasticity of kobeba was significantly ( $p \leq 0.05$ ) increased by marination process. **Burke and Mohnan (2003)** reported that the tenderizing action of acidic marinades is believed to involve several factors including weakening of structures due to swelling of the meat, increased proteolysis and increased conversion of collagen to gelatin. Lime

juice kobeba (2.30cm<sup>2</sup>/0.3g) followed by acetic acid kobeba (2.17cm<sup>2</sup>/0.3g) had higher ( $p \leq 0.05$ ) plasticity values than propionic acid kobeba (2.09cm<sup>2</sup>/0.3g). Plasticity of kobeba value did not significantly ( $P > 0.05$ ) change until one month of storage. While, from the second month of storage gradual decreases ( $P \leq 0.05$ ) in plasticity values were observed until the end of storage period. The reduction in plasticity of kobeba was supported by the increment in WHC values (reducing WHC).

The pH values of kobeba were significantly ( $p \leq 0.05$ ) decreased by marination process. Lime juice kobeba (pH 5.96) followed by acetic acid kobeba (pH 6.17) had lower ( $p \leq 0.05$ ) pH values than propionic acid kobeba (pH 6.27). Similar reduction in pH values of kobeba by marination process was reported by **Burke and Monahan (2003)** and **Sindelar et al (2003)**. The pH values of kobeba were gradually increased ( $P \leq 0.05$ ) during storage period. The increment might be attributed to the production of volatile basic components such as ammonia and total volatile nitrogen by meat spoilage bacteria (**Sallam et al 2007** and **Osheba, 2013**).

#### Cooking Characteristics of kobeba

Data in **Table (4)** showed that cooking loss values of kobeba were increased ( $p \leq 0.05$ ) after marination process. This increment was due to reduce the ability to bind water by lowering pH values. Lime juice kobeba had higher ( $p \leq 0.05$ ) cooking loss values than propionic acid and acetic acid kobeba.

**Carroll et al (2007)** reported that poultry meat with low pH has been associated with low water holding capacity, which results in increased drip loss and cook loss. Cooking loss value of kobeba did not significantly ( $P > 0.05$ ) change until one month of storage. While, from the second month of storage increases ( $P \leq 0.05$ ) in cooking loss values of kobeba were observed until the end of storage period.

Water and fat retention are related to the ability of protein matrix to retain water and bind fat (**Bochi et al 2008**). Water retention of kobeba did not significantly ( $P > 0.05$ ) change by marination process and during storage period. However, fat retention of kobeba was significantly ( $P \leq 0.05$ ) decreased by marination process but not by storage period ( $p > 0.05$ ). There is no significant ( $P > 0.05$ ) differences in fat retention of kobeba among marination process.

**Table 4.** Effect of marination type and storage at -18°C for 5 months on the cooking properties of kobeba

Treatment	Cooking loss (%)	Water retention (%)	Fat retention (%)
<b>Marination type</b>			
Unmarinated	4.65 <sup>d</sup>	74.82 <sup>a</sup>	131.4 <sup>a</sup>
lime juice	5.62 <sup>a</sup>	74.08 <sup>a</sup>	128.3 <sup>b</sup>
propionic acid	5.17 <sup>c</sup>	74.35 <sup>a</sup>	129.2 <sup>b</sup>
acetic acid	5.50 <sup>b</sup>	74.07 <sup>a</sup>	128.9 <sup>b</sup>
LSD	0.08	0.86	1.15
<b>Storage period (months)</b>			
0	5.02 <sup>e</sup>	74.11 <sup>a</sup>	129.44 <sup>a</sup>
1	5.09 <sup>e</sup>	74.21 <sup>a</sup>	129.45 <sup>a</sup>
2	5.18 <sup>d</sup>	74.27 <sup>a</sup>	129.46 <sup>a</sup>
3	5.28 <sup>c</sup>	74.37 <sup>a</sup>	129.47 <sup>a</sup>
4	5.40 <sup>b</sup>	74.47 <sup>a</sup>	129.48 <sup>a</sup>
5	5.50 <sup>a</sup>	74.55 <sup>a</sup>	129.49 <sup>a</sup>
LSD	0.08	1.05	1.41

Means in the same column with different letters are significantly different ( $p \leq 0.05$ )

#### Microbiological analysis of kobeba

Data in **Table (5)** showed that total bacterial counts of kobeba did not exceed the maximum levels 10<sup>5</sup>cfu/g of microbiological criteria for fresh and frozen poultry given by **Egyptian standard specifications (2009)**. Marinated kobeba had lower total bacterial and psychrophilic bacterial counts than control kobeba. These results are in agreement with those obtained by **Yusop et al (2010)** who found that the acidic marinating solutions decrease pH and inhibit microbial growth. Lime juice was more effective in reducing total bacterial and psychrophilic bacterial counts than acetic acid and propionic acid. This might be due to the strong antioxidant activity of bioactive compounds in lime juice. Lipolytic bacteria, *Salmonella Spp* and coliform bacteria were not detected in all kobeba. **Marwa (2014)** found that fresh breast chicken meat samples were completely free from coliform bacteria, lipolytic bacteria, *Salmonella spp* and yeast and mold, which proved the sanitary conditions of raw chicken breasts. Kobeba treated with lime juice, propionic acid and acetic acid had higher total yeast and mold counts than control kobeba. This might be due to the adaptation of the

**Table 5.** Microbiological analysis of kobeba as affected by marination type and storage at -18°C for 5 months

cfu/g	Storage period (months)	Marination type			
		unmarinated	lime juice	propionic acid	acetic acid
Total bacterial	0	8.77×10 <sup>4</sup>	3.21×10 <sup>4</sup>	6.71×10 <sup>4</sup>	5.10×10 <sup>4</sup>
	1	7.63×10 <sup>4</sup>	2.34×10 <sup>4</sup>	4.87×10 <sup>4</sup>	3.94×10 <sup>4</sup>
	2	6.94×10 <sup>4</sup>	2.05×10 <sup>4</sup>	4.12×10 <sup>4</sup>	3.66×10 <sup>4</sup>
	3	9.73×10 <sup>4</sup>	2.26×10 <sup>4</sup>	5.18×10 <sup>4</sup>	3.91×10 <sup>4</sup>
	4	6.96×10 <sup>5</sup>	3.89×10 <sup>4</sup>	6.60×10 <sup>4</sup>	4.81×10 <sup>4</sup>
	5	9.10×10 <sup>5</sup>	5.26×10 <sup>4</sup>	7.81×10 <sup>4</sup>	5.70×10 <sup>4</sup>
Psychrophilic bacteria	0	7.63×10 <sup>3</sup>	2.72×10 <sup>3</sup>	4.76×10 <sup>3</sup>	3.94×10 <sup>3</sup>
	1	6.96×10 <sup>3</sup>	2.24×10 <sup>3</sup>	4.36×10 <sup>3</sup>	3.26×10 <sup>3</sup>
	2	6.32×10 <sup>3</sup>	2.08×10 <sup>3</sup>	4.17×10 <sup>3</sup>	2.86×10 <sup>3</sup>
	3	7.42×10 <sup>3</sup>	3.24×10 <sup>3</sup>	5.24×10 <sup>3</sup>	3.52×10 <sup>3</sup>
	4	9.41×10 <sup>3</sup>	3.87×10 <sup>3</sup>	6.58×10 <sup>3</sup>	4.82×10 <sup>3</sup>
	5	3.27×10 <sup>4</sup>	5.41×10 <sup>3</sup>	7.97×10 <sup>3</sup>	6.63×10 <sup>3</sup>
Yeast and mold	0	1.63×10 <sup>2</sup>	2.35×10 <sup>2</sup>	1.95×10 <sup>2</sup>	2.25×10 <sup>2</sup>
	1	1.27×10 <sup>2</sup>	2.16×10 <sup>2</sup>	1.86×10 <sup>2</sup>	2.09×10 <sup>2</sup>
	2	0.92×10 <sup>2</sup>	1.92×10 <sup>2</sup>	1.53×10 <sup>2</sup>	1.78×10 <sup>2</sup>
	3	1.83×10 <sup>2</sup>	1.67×10 <sup>2</sup>	1.31×10 <sup>2</sup>	1.62×10 <sup>2</sup>
	4	2.16×10 <sup>2</sup>	2.02×10 <sup>2</sup>	1.67×10 <sup>2</sup>	1.97×10 <sup>2</sup>
	5	2.50×10 <sup>2</sup>	2.46×10 <sup>2</sup>	1.99×10 <sup>2</sup>	2.21×10 <sup>2</sup>

cfu/g = Colony forming units/gram, Lipolytic bacteria, *Salmonella Spp*, Coliform bacterial =Not detected

natural yeast and mold with lower pH values which found in marinated kobeba. These results are in agreement with those noted by **Ismail et al (2000)**.

Total bacterial and psychrophilic bacterial counts of all kobeba and yeast and mold of control kobeba were reduced until the second month of storage followed by slight increase up to the end of storage period. However, yeast and mold counts of marinated kobeba were reduced until the third month of storage followed by slight increase up to the end of storage period. The reduction of microorganism counts during 2-3 months of storage might be due to the breakdown of microorganisms cell wall by ice-crystals formed during freezing process. After these periods of storage, the increase in microorganism counts might be due to the increasing amino acids and fatty acids produced by hydrolysis of protein and fat during storage consequently lead to suitable conditions for growth of microorganisms. **Wally (2002)** reported that the increase in total bacterial counts after 5 to 6 months of storage might be due to development of

more cold tolerant viable cells of some spices originally existed.

#### Sensory evaluation of kobeb

Data in **Table (6)** showed that marinated kobeba had higher ( $p \leq 0.05$ ) rating scores for all sensory properties than control kobeba. This could be related to the function of organic acid, which degrades myofibrillar proteins and softens stromal proteins (**Burke and Monahan, 2003**). **Burke and Monahan (2003)** reported that the highest acceptance scores were for the golek chicken from marinating with citric acid.

Lim juice kobeba had higher ( $p \leq 0.05$ ) rating scores for all sensory properties than propionic acid and acetic acid kobeba. However, no significant ( $p > 0.05$ ) differences for all sensory properties were observed between propionic acid and acetic acid kobeba. Marinated kobeba had rating scores ranged between 7.32 and 7.98 for all sensory properties and described as like moderately.

**Table 6.** Effect of marination type and storage at -18°C for 5 months on the sensory properties of kobeba

Treatment	Taste	Odor	Color	Texture	Overall acceptability
<b>Marination type</b>					
unmarinated	6.29 <sup>c</sup>	6.32 <sup>c</sup>	6.35 <sup>c</sup>	6.78 <sup>c</sup>	6.41 <sup>c</sup>
lime juice	7.98 <sup>a</sup>	7.84 <sup>a</sup>	7.94 <sup>a</sup>	7.68 <sup>a</sup>	7.86 <sup>a</sup>
propionic acid	7.65 <sup>b</sup>	7.61 <sup>b</sup>	7.55 <sup>b</sup>	7.32 <sup>b</sup>	7.58 <sup>b</sup>
acetic acid	7.58 <sup>b</sup>	7.41 <sup>b</sup>	7.70 <sup>b</sup>	7.45 <sup>b</sup>	7.55 <sup>b</sup>
LSD	0.22	0.21	0.21	0.21	0.22
<b>Storage period (months)</b>					
0	8.26 <sup>a</sup>	8.21 <sup>a</sup>	8.18 <sup>a</sup>	8.10 <sup>a</sup>	8.17 <sup>a</sup>
1	8.01 <sup>a</sup>	8.03 <sup>a</sup>	7.94 <sup>a</sup>	7.89 <sup>a</sup>	7.97 <sup>a</sup>
2	7.60 <sup>b</sup>	7.67 <sup>b</sup>	7.60 <sup>b</sup>	7.55 <sup>b</sup>	7.61 <sup>b</sup>
3	7.31 <sup>c</sup>	7.28 <sup>c</sup>	7.31 <sup>c</sup>	7.20 <sup>c</sup>	7.28 <sup>c</sup>
4	6.81 <sup>d</sup>	6.60 <sup>d</sup>	6.88 <sup>d</sup>	6.74 <sup>d</sup>	6.76 <sup>d</sup>
5	6.25 <sup>e</sup>	6.19 <sup>e</sup>	6.45 <sup>e</sup>	6.43 <sup>e</sup>	6.33 <sup>e</sup>
LSD	0.27	0.26	0.26	0.26	0.27

Means in the same column with different letters are significantly different ( $p \leq 0.05$ )

However, control kobeba had rating scores ranged between 6.29 and 6.78 for all sensory properties and described as like slightly.

All sensory properties of kobeba were not significantly ( $P > 0.05$ ) change until one month of storage. While, gradual decreases ( $P \leq 0.05$ ) in all sensory properties of kobeba were observed from the second month of storage until the end of storage period. **Rasha (2011)** noticed that overall acceptability scores of chicken fingers were decreased by increasing the time of frozen storage.

### CONCLUSION

From the above results, it could be concluded that marinating spent hen meat in three different acidic solutions with 5% soy sauce for 30 minute at room temperature could increase water holding capacity values, plasticity, cooking loss, and all sensory properties of kobeba. Moisture content, total volatile nitrogen, fat retention, thiobarbituric acid, total bacterial count, psychrophilic bacteria and yeast and mold counts of kobeba were reduced. However, crude protein, crude fat, total ash and carbohydrates contents of kobeba were increased. Sensory properties of kobeba were reduced during storage period, but their rating scores described as like slightly or like moderately.

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