EFFECT OF DIFFERENT MARINATING SOLUTIONS ON MICROBIAL GROWTH INHIBITION AND QUALITY CHARACTERISTICS IMPROVEMENT OF BEEF SLICES DURING COLD STORAGE

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Abstract

The effectiveness of some marinating solutions on increasing the shelf life of beef slices stored at 4°C was investigated. Seven marinating solutions; constituting of one or more of the following: soy sauce, lime juice, propionic acid were used. 5.0 % spice mixture was added to each solution. It constituted of black pepper + onion and garlic powder + smoking liquid. The control sample was not soaked in any solution. Beef slices were soaked in the marinating solution for 15 min. The ratio of meat: solution was 1: 3 (w: v). The results indicated that the marinating solution T3 containing 95.0 % lime juice + 5.0 % spice mixture had the largest inhibition zone (using the disc assay procedure) towards *Escherichia coli.* Solution T4 constituting of 95.0 % propionic acid + 5.0 % spice mixture had the largest inhibition zone towards *Salmonella typhimurium.* In addition, the largest inhibition zone toward *Staphylococcus aureus* came from solution T2 constituting of 95.0 % soy sauce + 5.0 % spice mixture. The latter also produced the largest antimicrobial activity towards yeasts and molds present in meat samples. In addition, solution T3 had the best antimicrobial activity towards total plate count, psychrophiles, coliform and *Staphylococcus aureus* counts in meat samples and TVN and TBA values, but it had a bad water holding capacity (WHC), cooking loss and cooking yield. In addition, no salmonella growth was detected in all meat treatments at zero time and throughout the cold storage period. In conclusion, marinating solution T7; constituting of 31.67 % lime juice + 31.67 % propionic acid + 31.66 % soy sauce + 5.0 % spice mixture is the one recommended to be used commercially. It had a good antimicrobial activity, TVN, TBA, WHC, plasticity, cooking loss and cooking yield. In addition, the meat sample soaked in this solution had a shelf life of 15 days in the refrigerator at 4±1°C. Moreover, its sensory evaluation was highly superior.

INTRODUCTION

Meat is a highly favorable medium for growth of microorganisms, and it keeps well for only a short time at refrigeration temperatures. The short shelf- life of meat is attributed to its perishable nature, sanitation, practices during handling and time and temperature of storage. In addition, Zheng et al., (2005) reported that, the growth of microorganisms on meat is one of the main factors that causes discoloration and spoilage. Especially; in an environment of high relative humidity and insufficient air exchange, some microorganisms give rise to disagreeable odors and slime formation.

Furthermore, some microorganisms cause protein & fat degradation, changes in pigmentation, and in turn reduce shelf- life of meat at refrigeration temperature. Among meat microbial flora are *Escherichia coli, Staphylococcus aureus*, Salmonella and yeasts & molds (Buchanan and Gibbons, 1975).

In restaurants and hotels, meat is handled in a couple of ways. The first way is to store meat in the freezer; then, thaw it and refreeze it for several times; which would destroy its nutritional and quality attributes. The other way of handling meat is to store it in the refrigerator for two or three days until it is consumed. This allows microbial growth which leads to either spoilage of meat or to health problems that might lead to food poisoning. Hence, it is important to apply some treatments to refrigerated meat in order to inhibit microbial growth; and in the same time, improve the flavor and other quality attributes of meat. These treatments include dipping meat samples in different solutions (Burke and Monhan, 2003).

Also, Zheng et al., (2005) injected beef frankfurters with sodium diacetate and potassium benzoate which improved meat flavor. Along the same line, Sheard and Tali (2004) injected pork samples with sodium phosphate or sodium bicarbonate which resulted in raising the pH of pork to 5.75 – 5.97 compared to the pH of 5.45 for the control. This also increased cooking yield and decreased shear force. Also, sodium chloride or sodium tripolyphosphate increased cooking yield & tenderness, and decreased shear force for beef and pork samples (Pietrasik et al., 2005).

In this study, seven marinating solutions included one or more of the following components: Soy sauce, natural lime juice, propionic acid and mixture of black pepper, garlic and onion powder and smoking liquid were suggested to prolong the shelf- life of beef slices which have been stored under refrigeration condition .These constituents were used for their preservation or flavor effects. The objectives of this study were to determine the antibacterial activity of each marinating solution against *Escherichia coli, Staphylococcus aureus and Salmonella typhimurium* using the Disc Assay Procedure, and then evaluate the effect of soaking in different marinating solutions on the microbiological, chemical, physical and sensory properties of beef slices.

MATERIALS AND METHODS

Materials

Meat

Fresh lean beef from good grade carcasses was purchased from the private sector shop in the local market at Giza, Egypt. It was transferred to the laboratory in an ice box within 30 min.

Soy sauce

Soy sauce was obtained from Food Technology Research Institute, Soy Center, Agricultural Research Center, Giza, Egypt. It was prepared without the addition of caramel.

Lime juice

Lime juice was prepared by squeezing fresh lime fruits which were purchased from local market. Each liter of fresh lime juice was diluted by 2 liter of distilled water.

Propionic acid

Propionic acid (99.0% purity) is a product of Aldrich Chemical Company. It was obtained from Electrosient Company, Kasr El-Einy street Cairo, Egypt. Propionic acid concentration used in this study was 0.3 mol / liter.

Spices

The chosen spices which were selected to be used in marinating solutions i.e. black pepper, garlic and onion powder were obtained from local market.

Bacterial strains

Three strains of bacteria representing gram- negative bacteria, Escherichia *coli;* gram – positive bacteria, *Staphylococcus aureus;* and also the pathogenic bacteria strain *Salmonella typhimurium* were obtained from MIERCEN of the Faculty of Agriculture Ain shams University. These microorganisms were kept in our laboratory in the frozen state until used.

Methods

Technological methods

Preparation of marinating solutions

Table (1) shows the composition of marinating solutions. Each marinating solution was prepared by adding the spice mixture to the solution constituent. Then, the smoking liquid (obtained from the Unit of specific Nature, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt) was added to the mixture followed by continuous stirring until the spices were evenly distributed in the marinating solution. Then, marinating solutions were stored at 4 ºC for 24h before use.

Preparation of beef slices

The lean beef was boneless and trimmed of excess fat and connective tissue. Then, the beef was cut into slices weighing 100g with a size of about 10 × 7 × 2 cm. The beef slices were soaked in each marinating solution for 15 min at room temperature. The control sample was not soaked in any marinating solution. The ratio of meat: solution was 1: 3 (w / v). After marination, meat slices were placed in a strainer for 10 min in order to drain the solution off. Three slices of each treatment

were placed in polyethylene bags, and they were stored in the refrigerator (4±1°C). The polyethylene bags were held in single layers to ensure that each bag had similar exposure to surrounding air and light. Analyses were carried out at zero, 3, 6, 9, 12 and 15 day of refrigerated storage.

Analytical methods

Microbiological methods

Determination of antibacterial activity of different marinating solution

Activation of bacterial strains

Frozen cultures of *Staphylococcus aureus, Escherichia coli, and Salmonella typhimurium* were taken out of the freezer and kept at room temperature for one hour in order to get thawed. Two loopfuls of each culture were transferred aseptically into agar slants of Trypticase Soy, MacConkey and Brilliant Green, respectively. They were incubated at 37°C/ 24hr; then, they were kept in the refrigerator until used. To start work, cultures were activated by transferring two loopfuls from each agar slant into 9 ml of the broth specific for each microorganism. All inoculated broth media were incubated at 37°C/ 24hr. 0.1ml of each culture was inoculated into 9 ml of its respective broth medium and incubated at 37°C/ 24hr. The count of each culture was determined, followed by applying the Paper Disc Plate method.

Paper Disc Plate method

1.0 ml of the above incubated culture (average bacterial count for all strains $1.0 - 7.0 \times 10^6$ cfu /ml) was inoculated into 15 ml of sterile agar (45 – 50°C) specific for each organism. The inoculated agar was poured aseptically into sterile Petri plates. The agar was allowed to solidify. A sterile filter paper of 2.0 cm diameter was saturated with 150 ul of each marinating solution for 30 sec, and then it was placed in the center of each Petri plate containing the inoculated specific agar. The plates were incubated at 37°C/ 24hr. The diameter of each inhibition zone was determined in mm.

Microbial load

Total plate count (TPC), *Staphylococcus aureus*, Coliform group, Salmonellae , Psychrophilic and yeast & mold counts of beef slices were determined by using Nutrient, Trypticase Soy, MacConkey, Brilliant Green, Nutrient and Potato Dextrose Agar media, respectively according to the procedures described by Difco Manual (1984). Incubations were carried out at 37°C / 48hr for TPC ; at 37°C / 24hr for *Staphylococcus aureus*, Coliform and salmonellae ; at 7°C / 10 day for Psychrophilic and 25°C / 5 day for yeasts & molds counts.

Chemical analysis

Determination of pH

The pH value was measured by a pH meter (Jenway, 3510, UK) according to the method of Alken et al., (1962).

Storage stability

Total volatile nitrogen (TVN) was determined using the method published by Winton and Winton (1958). Thiobarbituric acid value (TBA) was estimated according to Pearson (1970).

Physical characteristics

Water holding capacity (WHC) and plasticity were measured according to the filter – press method of Soloviev (1966).

Cooking loss was determined after cooking in an oven at 180°C for 30 min as follows: -

[(Fresh sample weight – Cooked sample weight) / (Fresh sample weight)] × 100

% Cooking yield was determined as follows:

% Cooking yield = 100 – % Cooking loss.

Organoleptic evaluation

Organoleptic evaluation of cooked beef slices was carried out according to Watts et al., (1989) by aid of 10 panelists. Judging scale was as follows:

Very good	8 – 9
Good	6 – 7
Fair	4 – 5
Poor	2 – 3
Very poor	0 – 1

Statistical analysis

Data of Organoleptic evaluation were subjected to analysis of variance (ANOVA). Means comparison was performed using Duncan's test at the 5% level of probability as reported by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Antibacterial activity of different marinating solutions.

Data in Table (2) show the antibacterial effect of different marinating solutions against two gram negative bacterial strains (*Escherichia coli, and Salmonella typhimurium*) and one gram positive bacterial strain (*Staphylococcus aureus*), expressed as the diameters of inhibition zones (mm). The results showed that, marinating solution which prepared with 95.0 % lime juice + 5.0 % spices mixture (T3) had the largest inhibition zone against *E coli,* followed by T4 (95.0% propionic acid + 5.0 % spices mixture , that was due to the undissociated acid molecules which

are responsible for preservative effect of organic acids (Francis, 2000). Also, the citrus bioflavonoid had antimicrobial properties. These compounds have reportedly wide-range antimicrobial properties effective against a broad range of human pathogenic, fungi and food spoilage organisms (Fernandez-Lopez et al., 2005).

The smallest inhibition zone (26 mm) was observed for marinating solution T1 which contained 5.0 % spices mixture only. On the contrary, marinating solution no. T4 (95.0 % Propionic acid + 5.0 % spice mixture) had the largest inhibition zone (32 mm) against *Salmonella typhimurium* followed by T7. Again, the smallest inhibition zone (22 mm) was noticed for marinating solution which contained 5.0 % spices mixture only (T1).

Concerning *Staphylococcus aureus,* the largest inhibition zone (37 mm) was recorded for marinating solution T2, followed by T5 and then T7. This indicated that soy sauce was the strongest antimicrobial substance against *Staphylococcus aureus.* Probably, that was due to the lack of soy sauce to the essential amino acid methionine (Francis, 2000). The latter might be necessary for the respiration of *Staphylococcus aureus,* and its lack might have caused death to this microorganism. This is supported by Buchanan and Gibbons (1975) who reported that endogenous respiration of *Staphylococcus aureus* occurs by the utilization of free amino acids within the cell pool, and that this organism required up to 12 amino acids for aerobic growth. Finally, The smallest inhibition zone (25 mm) came from marinating solution T1.

Effect of marinating solutions on pH value of beef slices.

Concerning Table (3), it could be seen that the pH of control treatment at zero time presents the pH of fresh meat. It is clear that, the quality of meat was good, because its pH was high (pH = 6.08). This pH is far from the iso electric point (IEP) of meat proteins (pH = 5.5) which enables the meat proteins to bind large number of water molecules. Treatment T1 came in the second order of pH value (pH = 5.72). That was probably due to the acidic effect of liquid smoke present in the spice mixture. In addition, the lowest pH value was recorded for T3 (pH = 4.75), which was soaked in a natural lime juice solution plus spice mixture. Although, the higher pH value of the meat is the better (Francis, 2000); the low pH value still could have other benefits such as an antimicrobial activity and a tenderizing effect (Burke and Monahan, 2003).

By advancement of cold storage time, the pH values of all treatments were decreased. Probably, that was due to the drip that took place during storage .The latter resulted in the loss of small protein molecules, which have a buffering effect. In addition, the growth of the useful microorganisms (Lactic acid bacteria) lead also to decreased pH values (Buchanan and Gibbons 1975). Moreover, this decrease of pH values in all treatments during cold storage may be due to the breakdown of glycogen to produce lactic acid and consequently decreased pH value.

Effect of marinating solutions on microbial load of beef slices.

From Table (4) it could be seen that, at zero time the lowest total plate count (TPC) was recorded for treatment T3 followed by T7. These treatments had low pH values (4.75 and 4.95, respectively, Table 3) which were responsible for reducing the microbial growth. On the other hand, the highest TPC came from control sample (C) followed by T1. They had higher pH values (6.08 and 5.72, respectively) which allowed more microbial growth. However, all treatments did not reach the rejection level of TPC (1.0×10^6 cfu /g, Egyptian Standard, 2005).

With refrigerated storage, TPC values for all treatments were increased, due to the continuous microbial growth .Treatments T3, T4 and T7 had a shelf life of 15 day. However, treatments T2, T5 and T6 had a shelf life of only 12 day. On the other hand, treatment T1 and control sample had a shelf life of 9 day.

Concerning coliform bacterial growth, at zero time, it is obvious that the lowest growth came from treatment T3 followed by T4, then T7; and the highest count was recorded for control sample followed by T1 then T2. Again, the coliform growth was dependent on pH values. With refrigerated storage, coliform count was increased for all treatments; however, the lowest growth was observed for treatment T3 followed by T7 at the end of cold storage (15 day).

Staphylococcus aureus growth at zero time was the lowest for treatment T3 followed by T4, then T7 and was the highest for control sample followed by T1. Again, with storage, *Staphylococcus aureus* was progressively increased as the period of cold storage increased for all treatments with the lowest growth for treatment T3 followed by T7 at the end of cold storage (15 day).

The psychrophilic microbial growth, at zero time, was the lowest for treatment T3 followed by T4, then T7; and was the highest for control sample followed by T1, then T2. Again, by prolongation of cold storage, all treatments had the psychrophilic microbial growth increased with the lowest growth for treatment T3 followed by T4 and then T7 at the end of cold storage (15 day).

Concerning Salmonellae growth, no colonies were detected for all treatments at either zero time or along with cold storage period.

From the data in table (5) it could be noticed that, the highest growth of yeasts & molds, at zero time was observed for treatment T4, T5 and T7 which had pH values in the range of 4.95 – 5.05 (Table 3). On the contrary, T2, control sample (C), T1, and T6 which had pH values in the range of 5.22 – 6.08, revealed lower yeast & mold growth. In addition, treatment T3 also gave low growth and had a pH of 4.75. From the above, it could be suggested that the natural yeast and mold flora that were present in meat samples had possibly an optimum pH value of 5.0.

With refrigerated storage, all treatments had the growth of yeasts & molds increased with the highest growth at the end of cold storage (15 day) for treatment T3 followed by T4, then T7. The latter had pH values in the range of 4.47 – 4.50 (Table 3). This contradicts with the above finding that the highest yeast & mold growth was around pH 5.0. This might indicate the adaptation of the natural yeast & mold flora to lower pH values

In conclusion of Tables (4 and 5); except for yeast & mold growth at zero time, treatment T3 followed by T7 gave the lowest count and control sample followed by T1 gave the highest one for all types of microbial growth. This coincides with results of table (6) where T7 followed by T3 revealed the lowest TVN value and control sample C showed the highest one at zero time or along with cold storage period. Furthermore, treatment T3 followed by T7 also had the lowest count for most types of microbial growth at the end of cold storage (at 15 day). This coincides with data of table (6) where treatment T3 had the lowest TBA value at 15 day of cold storage.

Effect of marinating solutions on storage stability of beef slices.

From Table (6), it could be noticed that, at zero time, treatment T7 had the lowest total volatile nitrogen (TVN) and control sample C had the highest level. This was due to the low and high pH values of these treatments, respectively (Table 3). It is known that TVN value is a result of protein breakdown, due to microbial activity, which produce basic nitrogenous compounds. Since microbial activity need pH values close to neutrality, high pH lead to a large amount of protein breakdown, and consequently to the highest TVN value for control sample. However, all TVN values at zero time were still far away from the rejection level (20 mg / 100g) as reported by Egyptian Standard (2005).

During cold storage the total volatile nitrogen in all treatments progressively increased as the time of cold storage increased. This was due to the microbial breakdown activity which confirmed by the rapid development in TVN. However, meat treatments T3, T4, T5, T6 and T_7 had TVN values less than 20 mg / 100g until 12 and 15 (for T7 only) days of cold storage. But, treatments T_2 and both control sample C and T_1 had TVN values less than rejection level only until 9 and 6 days respectively.

Concerning TBA values at zero time, they were all very low (TBA: 0.109 – 0.128 mg malonaldhyde / kg).With refrigeration storage, TBA values were progressively increased as the period of storage increased for all treatments due to the penetration of oxygen though the polyethylene bags in which treatments samples were stored . This lead to lipid oxidation by the action of meat enzymes or microbial activity. At 9 day of cold storage, all treatments had TBA values lower than the

rejection level (0.9 mg / kg) as reported by Egyptian Standard (2005), except for control sample C which had a TBA value of 1.379 mg /kg. This shows good antioxidant effect for all marinating solutions, with treatments T4 and T6 (containing propionic acid) having the least antioxidant activity. At the end of cold storage (15 day) all treatments reached the rejection level, except treatments T_3 and T_5 (containing lime juice) which was due to the strong antioxidant activity of bioactive compounds (flavonoids, ascorbic acid and citric acid). Ascorbic acid, a well known natural antioxidant, together with natural flavonoids are also attracting not only due to their antioxidant properties, but also as anti-carcinogenic and anti-inflammatory agents because of lipid anti-peroxidation effects (Martin et al., 2002).

Effect of marinating solutions on physical properties of beef slices.

From Table (7), it is clear that, the best water holding capacity score (WHC) at zero time was for treatment T2 followed by control sample C, then T1. That was due to the ability of meat protein molecules to bind water. The worst WHC score was observed for treatment T3 which was soaked in a solution containing 95.0 % lime juice + 5.0 % spice mixture. This possibly resulted from low pH (Table 3) leading to decreased ability to bind water. Propionic acid (treatment T4) revealed a close effect to lime juice which resulted also in a bad WHC probably due to low pH. Concerning treatments T5, T6 and T7 they had a better WHC due to the effect of soy sauce proteins which increased the ability to bind water.

With refrigerated storage, WHC deteriorated for all treatments. That was due to the drip that took place during refrigerated storage. The latter resulted in some loss of small protein molecules (water soluble) which lead to a worse WHC scores (Francis, 2000) and possibly decline of pH .Treatments T2 and T6 had the best WHC scores after 15 day of cold storage respectively; in these samples pH values were comparatively high.

The best plasticity at zero time was for treatment T3 followed by T7. That was due to possible swelling and solubilisation of collagen by the effect of either lime juice alone or both lime juice and propionic acid, respectively. This is supported Burke and Monahan (2003) who 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 by cathepsins and increased conversion of collage to gelatin at low pH during cooking. With advancement of refrigerated storage time, the plasticity for all treatments progressively deteriorated. That was, again, due to the drip that took place which lead to the loss of water during refrigerated storage. The best plasticity score after 15 day of cold storage was recorded for treatment T3.

Concerning cooking loss and cooking yield, at zero time, it is clear that the best values were recorded for treatment T2 which coincided with having the best WHC too. This was due to the presence of soy sauce proteins in addition to meat proteins. Both of the latter bound water molecules, improved WHC and consequently, enhanced cooking loss decrease and cooking yield increase. Control sample C came in the second order of cooking loss and cooking yield followed by T1 which coincided too with WHC. The worst values were for treatment T3 which also coincided with having the worst WHC. In addition, treatment T7 had a moderate cooking loss and cooking yield, and a moderate WHC too.

Effect of marinating solutions on sensory properties of beef slices.

From the data in Table (8), it could be observed that, sensory properties of beef slices i.e., taste, odor, texture and overall acceptability were significantly affected by constituents of marinating solutions. Concerning taste and odor, treatment T3, T5 and T7 were the best with no significant differences between them. Hence, lime juice had the best taste and odor among additives used in the marinating solutions. Furthermore, treatments T2, T4 and T6 showed nonsignificant differences, and were worse than the previous treatments. So, the consumer preference for soy sauce and propionic acid were similar and were less accepted than lime juice.

The texture was the best for treatments T3, T4, T5, T6 and T7 which were nonsignificantly different from each other. That was probably due to the presence of lime juice (citric acid) and propionic acid in marinating solutions. This lowered pH values of meat which helped in swelling of collagen process and produced tender meat samples. This is supported Burke and Monahan (2003).

Concerning color evaluation, treatment T3 was the best among other treatments. This was probably due to the antioxidant effect of lime juice which protected meat color against oxidation. Treatments T2, T4, T5, T7 and T1 showed nonsignificantly different scores, and came in the second order after T3.Again, this was possibly due to the antioxidant effect of lime juice. The worst treatment was control sample C which was probably due to the lack of any antioxidant effect because it was not soaked in any marinating solution.

Treatments T3, T5 and T7 scores were nonsignificantly different and they had the best overall acceptability which was expected as these samples had already the best taste, odor, texture and color. This indicated the preference of judges to treatments samples containing lime juice. In the second order of overall acceptability came from treatments T2, T4 and T6 (containing soy sauce, propionic acid and mixture of them respectively) which had nonsignificant differences. Furthermore, control sample had the lowest overall acceptability.

CONCLUSION

Marinating solution T7 is the one recommended to be used commercially. Although T3 had the best antibacterial activity, TVN and TBA values; however, it had a bad WHC, cooking loss and cooking yield. Hence, the most preferred marinating solution is that of treatment T7. That is because it had the second order in the antibacterial activity for most types of microorganisms present in meat samples (except for yeasts & molds). Furthermore, it also had the second order in reducing the values of TVN and TBA. Moreover, it showed a moderate WHC, cooking loss and cooking yield and had the second order in plasticity. In addition, its sensory evaluation was highly superior.

Table 1. Formulations of marinating solutions.

Marinating solution No.	Constituents of marinating solutions (%)
T_1	95.0 Dis. water + 5.0 spice mix.*
T_2	95.0 Soy sauce + 5.0 spice mix.
T_3	95.0 Lime juice + 5.0 spice mix.
T_4	95.0 Propionic acid + 5.0 spice mix.
T_5	50.0 Lime juice + 45.0 soy sauce + 5.0 spice mix.
T_6	50.0 Propionic acid + 45.0 soy sauce + 5.0 spice mix.
T_7	31.67 Lime juice + 31.67 propionic acid +31.66 soy sauce + 5.0 spice mix.

*5 g spice mix. constituted of 1.9 g onion powder + 1.5 g garlic powder + 0.6 g black pepper + 1.0 ml smoking liquid.

Table 2. Diameters of inhibition zones (mm) of different marinating solutions.

Treatments / Microorganisms	T_1	T_2	T_3	T_4	T_5	T_6	T_7
E. coli	26	29	55	53	42	38	40
Sal. typhimurium	22	26	28	32	24	27	30
Staph. aureus	25	37	30	27	34	28	32

Table 3. PH values of beef slices soaked in different marinating solutions during refrigerated Storage.

Treatments / Storage period (Days)	C	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Zero-time	6.08	5.72	5.45	4.75	5.02	5.05	5.22	4.95
3	5.84	5.65	5.43	4.63	4.99	4.91	5.03	4.83
6	5.70	5.50	5.38	4.60	4.82	4.80	4.89	4.79
9	5.63	5.41	5.29	4.55	4.71	4.72	4.76	4.68
12	-*	-*	5.20	4.50	4.63	4.65	4.71	4.63
15	-*	-*	-*	4.47	4.57	4.60	4.62	4.50

*PH value was not measured because meat samples were spoiled.

C: Control.

Table 4. Bacterial growth in beef slices soaked in different marinating solutions during refrigerated storage.

Treatments / Storage period (Days)	C	T_1	T_2	T_3	T_4	T_5	T_6	T_7
	Total plate count (cfu / g)							
Zero-time	$7.85x10^3$	$7.20x10^3$	$1.23x10^3$	$3.85x10^2$	$6.25x10^2$	$7.45x10^2$	$8.95x10^2$	$4.55x10^2$
3	$5.62x10^4$	$2.35x10^4$	$8.25x10^3$	$8.70x10^2$	$1.79x10^3$	$3.85x10^3$	$6.60x10^3$	$9.10x10^2$
6	$1.95x10^5$	$8.95x10^3$	$2.74x10^4$	$2.73x10^3$	$7.15x10^3$	$9.30x10^3$	$1.65x10^4$	$6.55x10^3$
9	$9.60x10^5$	$5.10x10^5$	$1.82x10^5$	$9.35x10^3$	$5.25x10^4$	$7.65x10^4$	$8.75x10^4$	$3.28x10^4$
12	-*	-*	$9.35x10^5$	$5.25x10^4$	$1.50x10^5$	$5.20x10^5$	$7.95x10^5$	$1.15x10^5$
15	-*	-*	-*	$4.70x10^5$	$9.27x10^5$	$1.07x10^6$	$1.13x10^6$	$8.80x10^5$
	Coliform group (cfu / g)							
Zero-time	$9.5x10^1$	$8.5x10^1$	$7.5x10^1$	$2.5x10^1$	$3.50x10^1$	$5.00x10^1$	$5.50x10^1$	$4.00x10^1$
3	$1.45x10^2$	$1.05x10^2$	$9.5x10^1$	$4.00x10^1$	$7.50x10^1$	$8.50x10^1$	$9.00x10^1$	$6.00x10^1$
6	$1.85x10^2$	$1.70x10^2$	$1.15x10^2$	$7.50x10^1$	$1.30x10^2$	$1.50x10^2$	$1.65x10^2$	$9.50x10^1$
9	$2.05x10^2$	$1.90x10^2$	$1.55x10^2$	$1.25x10^2$	$1.65x10^2$	$1.85x10^2$	$1.95x10^2$	$1.45x10^2$
12	-*	-*	$2.10x10^2$	$1.75x10^2$	$1.90x10^2$	$2.20x10^2$	$2.35x10^2$	$1.85x10^2$
15	-*	-*	-*	$2.15x10^2$	$2.45x10^2$	$2.65x10^2$	$2.90x10^2$	$2.30x10^2$
	Staphylococcus aureus (cfu / g)							
Zero-time	$1.95x10^2$	$1.80x10^2$	$1.50x10^1$	$0.75x10^1$	$0.80x10^2$	$1.05x10^2$	$1.45x10^2$	$0.95x10^2$
3	$4.50x10^2$	$4.10x10^2$	$3.05x10^2$	$1.10x10^2$	$1.55x10^2$	$1.85x10^2$	$2.30x10^2$	$1.60x10^2$
6	$9.85x10^2$	$7.95x10^2$	$7.25x10^2$	$2.85x10^2$	$3.50x10^2$	$3.95x10^2$	$4.25x10^2$	$3.15x10^2$
9	$1.35x10^3$	$1.12x10^3$	$9.05x10^2$	$4.90x10^2$	$5.35x10^2$	$7.40x10^2$	$7.85x10^2$	$6.60x10^2$
12	-*	-*	$10.03x10^2$	$6.55x10^2$	$7.80x10^2$	$8.90x10^2$	$9.70x10^2$	$8.20x10^2$
15	-*	-*	-	$8.85x10^2$	$9.60x10^2$	$1.09x10^3$	$1.22x1^3$	$9.25x10^2$
	Psychrophilic bacteria (*cfu / g*)							
Zero-time	$9.25x10^2$	$6.65x10^2$	$5.30x10^2$	$0.95x10^2$	$1.15x10^2$	$3.85x10^2$	$3.50x^2$	$1.55x10^2$
3	$7.90x10^3$	$4.25x10^3$	$1.85x10^3$	$2.25x10^2$	$7.20x10^2$	$9.35x10^2$	$8.85x$	$9.75x10^2$
6	$3.20x10^4$	$9.35x10^3$	$6.75x10^3$	$8.75x10^2$	$2.53x10^3$	$2.11x10^3$	$5.95x10^3$	$4.85x10^3$
9	$9.85x10^4$	$7.65x10^4$	$3.70x10^4$	$4.85x10^3$	$6.75x10^3$	$9.65x10^3$	$2.27x10^4$	$8.15x10^3$
12	-*	-*	$8.65x10^4$	$1.12x10^4$	$2.95x10^4$	$5.70x10^4$	$7.25x10^4$	$3.50x10^4$
15	-*	-*	-*	$7.35x10^4$	$8.15x10^4$	$9.85x10^4$	$1.02x10^5$	$9.25x10^4$
	Salmonella growth							
At zero - time and during storage	ND**	ND	ND	ND	ND	ND	ND	ND

*Microbial growth was not determinated because meat samples were spoiled.

** No growth was detected. C: Control.

Table 5. Yeasts and molds counts (cfu / g) of beef slices soaked in different marinating solutions during cold storage.

Treatments / Storage period (Days)	C	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Zero-time	1.5×10^1	1.5×10^1	1.0×10^1	1.5×10^1	2.0×10^1	2.0×10^1	1.5×10^1	2.0×10^1
3	4.0×10^1	3.0×10^2	2.0×10^1	6.5×10^1	5.0×10^1	3.0×10^1	2.5×10^1	4.5×10^1
6	7.5×10^1	4.5×10^2	3.5×10^2	9.0×10^1	7.5×10^1	6.5×10^1	5.0×10^1	8.0×10^1
9	1.3×10^2	6.5×10^2	4.0×10^2	1.25×10^2	1.05×10^2	8.0×10^1	7.0×10^1	9.5×10^1
12	-*	-*	5.5×10^2	1.5×10^2	1.2×10^2	9.5×10^1	9.0×10^1	1.35×10^2
15	-*	-*	-*	1.9×10^2	1.85×10^2	1.25×10^2	1.1×10^2	1.7×10^2

* Yeasts and molds counts were not determinated because meat samples were spoiled.

C: Control.

Table 6. Total volatile nitrogen (TVN) and thiobarbituric acid (TBA) values of beef slices soaked in different marinating solutions during cold storage.

Treatments / Storage period (Days)	C	T_1	T_2	T_3	T_4	T_5	T_6	T_7
	TVN (mg / 100g)							
Zero-time	11.90	11.48	11.62	11.06	11.34	11.20	11.20	10.92
3	15.40	14.28	12.60	12.18	14.56	12.88	13.30	11.76
6	18.62	16.80	15.40	14.00	15.82	14.70	15.12	13.72
9	22.12	20.72	18.62	16.24	18.48	16.66	17.92	15.82
12	-*	-*	20.72	16.20	19.32	18.76	18.90	17.08
15	-*	-*	-*	20.30	24.64	21.70	23.10	19.60
	TBA (mg malonaldehyde / kg)							
Zero-time	0.121	0.119	0.111	0.109	0.129	0.120	0.128	0.109
3	0.579	0.311	0.290	0.180	0.369	0.259	0.329	0.309
6	0.759	0.390	0.350	0.269	0.489	0.320	0.459	0.379
9	1.379	0.589	0.680	0.500	0.790	0.620	0.750	0.560
12	-*	-*	0.919	0.690	0.979	0.759	0.969	0.869
15	-*	-*	-*	0.849	1.209	0.869	1.089	0.979

* TVN and TBA values were not measured because meat samples were spoiled.

C: Control.

Table 7. Water holding capacity (WHC) and plasticity scores of beef slices soaked in different marinating solutions during cold storage.

Treatments / Storage period (Days)	C	T_1	T_2	T_3	T_4	T_5	T_6	T_7
	WHC (cm^3 / 0.3g)							
Zero-time	2.75	2.85	2.30	5.00	4.30	3.30	3.00	3.65
3	3.20	3.00	2.80	5.50	4.60	3.70	3.35	4.20
6	3.85	3.60	3.35	5.90	5.10	4.15	3.80	4.65
9	5.30	4.20	3.85	6.50	5.80	4.65	4.40	5.15
12	-*	-*	4.40	7.30	6.30	4.95	4.70	5.80
15	-*	-*	-*	7.90	6.70	5.60	5.20	6.35
	Plasticity (cm^3 / 0.3g)							
Zero-time	1.65	1.80	1.95	2.85	2.25	2.20	2.00	2.50
3	1.50	1.70	1.80	2.70	2.00	2.00	1.95	2.20
6	1.40	1.60	1.70	2.45	1.85	1.90	1.70	1.90
9	1.25	1.40	1.45	2.30	1.60	1.70	1.50	1.60
12	-*	-*	1.30	2.10	1.45	1.50	1.35	1.40
15	-*	-*	-*	1.95	1.35	1.40	1.20	1.30
	Cooking loss (%)							
Zero-time	22.41	23.20	20.83	33.20	31.90	27.52	25.90	29.05
	Cooking yield (%)							
Zero-time	77.59	76.80	79.17	66.80	68.10	72.48	74.10	70.95

* WHC and plasticity values were not measured because meat samples were spoiled.

C: Control.

Table 8. Sensory evaluation of beef slices soaked in different marinating solutions at zero time.

Sensory properties / Treatments	Taste	Odor	Texture	Color	Overall acceptability
Control (C)	6.90^{e}	7.00^{d}	6.80^{b}	6.60^{d}	6.82^{e}
T1	7.50^{d}	7.60^{c}	7.00^{b}	7.80^{bc}	7.47^{d}
T2	8.10^{bcd}	8.00^{bc}	7.00^{b}	7.90^{bc}	7.75^{cd}
T3	8.70^{ab}	8.70^{a}	8.80^{a}	8.90^{a}	8.77^{a}
T4	8.00^{cd}	7.90^{bc}	8.50^{a}	8.30^{b}	8.17^{bc}
T5	8.80^{a}	8.30^{ab}	8.60^{a}	8.10^{bc}	8.45^{ab}
T6	8.60^{abc}	8.00^{bc}	8.20^{a}	7.50^{c}	8.07^{bc}
T7	8.70^{ab}	8.60^{a}	8.40^{a}	8.20^{b}	8.47^{ab}
LSD at 0.05 level	0.57^{***}	0.54^{***}	0.79^{***}	0.55^{***}	0.321^{***}

Where: Mean values in the same column with the same letter are not significantly different at 0.05 level.
*** High significant.

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EFFECT OF DIFFERENT MARINATING SOLUTIONS ON MICROBIAL GROWTH INHIBITION AND QUALITY CHARACTERISTICS IMPROVEMENT OF BEEF SLICES DURING COLD STORAGE

تأثير محاليل النقع المختلفة على تثبيط النمو الميكروبى وتحسين خصائص جودة شرائح اللحم البقرى أثناء التخزين بالتبريد

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لقد تم دراسة كفاءة بعض محاليل النقع المقترحة على اطالة مدة حفظ شرائح اللحم البقرى المخزنة على درجة ٤ْم. وقد تم استخدام سبعة أنواع من محاليل النقع المختلفة.وقد تكونت هذه المحاليل من واحد أو أكثر من المكونات التالية : صوص صويا ، عصير ليمون أو حمض بروبيونيك. ولقد تم اضافة ٥ % خلطة توابل لكل محلول. وتكونت خلطة التوابل من فلفل اسود + بودره بصل و ثوم + سائل تدخين. وعينة الكنترول لم يتم غمرها فى أى من محاليل النقع السابقة. وقد تم غمر شرائح اللحم فى أى محلول لمدة ١٥ دقيقة وكانت نسبة اللحم : المحلول هى ١: ٣ (وزن : حجم).

ولقد وجد أن محلول النقع المكون من ٩٥ % عصير ليمون + ٥ % مخلوط توابل قد أدى الى أكبر دائرة خالية من النمو الميكروبى (مساحة التثبيط) ضد بكتيريا *Escherichia coli* . وكذلك وجد أن محلول النقع المكون من٩٥ % حمض بروبيونيك + ٥ % مخلوط توابل أدى الى تكون أكبر مساحة تثبيط من النمو الميكروبى ضد بكتيريا *Salmonella typhymurium* وبالاضافة لما سبق، فان أكبر مساحة تثبيط ضد بكتيريا *Staphylococcus aureus* قد نتجت من محلول النقع المكون من ٩٥ % صوص صويا + ٥ % مخلوط توابل وكذلك فان هذا المحلول قد أعطى أكبر نشاط مضاد للميكروبات ضد الخمائر و الفطريات الموجودة فى عينات اللحم. بالاضافة لما سبق، فان محلول النقع المكون من ٩٥ % عصير ليمون + ٥ % مخلوط توابل قد أعطى أفضل نشاط مضاد للميكروبات الموجودة فى عينات اللحم وذلك ضد أعداد البكتيريا الكلية ، البكتيريا المحبة للبرودة ، مجموعة الكوليفورم ، وبكتيريا ستافيلوكوس اورياس. وكذلك أعطى هذا المحلول أفضل قيم للنيتروجين الكلى المتطاير ولحمض الثيوباربيتوريك. ولكن هذا المحلول أدى الى تقليل قدرة اللحم على الاحتفاظ بالماء وعائد الطهى وزيادة الفقد بالطهى. وعلاوه على ماسبق، فلم توجد هناك أى نموات لبكتيريا السالمونيلا فى كل عينات اللحم سواء العينات المعاملة بمحاليل النقع المختلفة أو عينة الكنترول الغير معامل وذلك بعد النقع مباشرة وأثناء التخزين بالتبريد.

هذا وقد تم التوصيه باستخدام محلول النقع المكون من ٣١,٦٧% عصير ليمون + ٣١,٦٧% حمض بربيونيك + ٣١,٦٦% صوص صويا حيث أن هذا المحلول قد أدى الى قدره عالية مضاده للميكروبات فى عينات اللحم. وأعطى قيم جيدة للنيتروجين الكلى المتطاير، وحمض الثيوباربيتوريك و القدره على الاحتفاظ بالماء و البلاستكيه والفقد بالطهى وعائد الطهى. كذلك فان عينات اللحم المنقوعة فى هذا المحلول كان لها مده حفظ مقدارها ١٥يوم فى الثلاجة . بالاضافة الى ذلك ، فان التقييم الحسى لهذا اللحم كان ممتازا .