International Association for
Food Protection®
6200 Aurora Avenue, Suite 200W
Des Moines, Iowa 50322-2864, USA

Certificate of Attendence

Ugur Gogus
Middle East Technical University
Vocational School of Higher Education
Ankara 06531
Turkey

This certifies that:

Ugur Gogus

has attended IAFP 2005, the Association's 92nd Annual Meeting held August 14-17, 2005 in Baltimore, Maryland and presented, T4-06, "A Comparative Study as Related to the Effects of Glucose Monohydrate, Hot Water and Sodium Pyrophosphate on Some Quality Criteria of Deboned and Matured Brisket".

Thank you.

Julie Cattanach
Meeting Coordinator

T4-06 A Comparative Study as Related to the Effects of Glucose Monohydrate, Hot Water and Sodium Pyrophosphate on Some Quality Criteria of Deboned and Matured Brisket

UGUR GOGUS, Faruk Bozoglu, and Hami Alpas, Middle East Technical University, Vocational School of Higher Education, Ankara, Turkish Republic, 06531, Turkey

Organic acids, hot water and chlorine have been commonly used in carcass decontamination for years. Organic acids had adverse effects on color and were corrosive, while high temperature water was discoloring. On the contrary, glucose fermentation by lactic acid bacteria in meat during rigor might be effective in microbial inhibition without adverse effect. Maturation of beef brisket primal cuts occurred for 2 days at 11°C and then at 5°C for 4 days in 85% relative humidity. Briskets were dipped in D(+) Glucose Monohydrate (GM) (16.51 g/100 ml, 15%) on the 7th. day of this maturation. Other primals were treated by hot water immersion (HW); Sodium Pyrophosphate (SPP) and HW; GM+SPP+HW; or GM+HW. 1.63 and 1.41 log reductions in coliforms 1.90 and 1.80 log reductions in *Pseudomonas* spp., and 0.83 and 0.72 log reductions in MAB (Mesophile Aerobic Bacteria) were observed in GM+HW and SPP+GM+HW respectively at day 8 of shelf life under 4°C, indicating a significant protective effect of the concerned applications with their lowest acidity losses, while SPP's increasing effect of heat penetration was not detected during pasteurization, from the standpoint of *Pseudomonas* and coliform inhibitions ($P < 0.05$ and $P < 0.01$).

International Association for
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6200 Aurora Avenue, Suite 200W
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May 24, 2005

Dr. Ugur Gogus
Middle East Technical University
Vocational School of Higher Education
Ankara, Turkish Republic
06531 Turkey

Dear Dr. Gogus,

This letter is written to officially invite you to participate at the IAFP 2005 – the Association's 92nd Annual Meeting, August 14-17, 2005 at the Baltimore Marriott Waterfront Hotel in Baltimore, Maryland U.S.A. The intent of this letter is to assist you in obtaining a visa to allow your attendance at IAFP 2005. Please note that attendees are responsible for all travel and registration expenses incurred to attend the meeting.

Our Meeting is a gathering of Food Safety professionals and interested persons from around the world. It is an excellent forum to learn about the latest developments and techniques in food science and food safety.

We hope that you will be able to attend our Food Safety Conference and present your paper titled "A Comparative Study as Related to the Effects of Glucose Monohydrate, Hot Water and Sodium Pyrophosphate on some Quality Criteria of Deboned and Matured Brisket" program #T4-06, which will be presented at the IAFP 2005 meeting.

If I can be of further assistance, please contact me at the Association office.

Sincerely,

David W Tharp

David W. Tharp, CAE
Executive Director

International Association for
Food Protection®
6200 Aurora Avenue, Suite 200W
Des Moines, Iowa 50322-2864, USA

May 13, 2005

Ugur Gogus
Middle East Technical University
Vocational School of Higher Education
Ankara, Turkish Republic 06531
Turkey

Dear Ugur:

Your participation at IAFP 2005, August 14-17, in Baltimore, Maryland, is important to the success of the Meeting. Following is information regarding your presentation, including the title and presentation time. Enclosed is a copy of your abstract that will appear in the Program Book. **Please note that there may have been editorial changes made to your abstract.** Review this information carefully. Notify me immediately if you detect an error.

Session Title: Antimicrobials

Title of Presentation: T4-06 - A Comparative Study as Related to the Effects of Glucose Monohydrate, Hot Water and Sodium Pyrophosphate on Some Quality Criteria of Deboned and Matured Brisket

Your presentation has been scheduled for: Tuesday from 2:45 p.m. to 3:00 p.m.

Your speaking time includes questions and answers! At the end of the allotted time, the room lights will be turned on and you will be expected to conclude your presentation. Prior to the start of your technical session contact your convener, let them know you are present, and provide them with any details concerning your presentation. LCD projectors will be provided again this year; slide projectors will not be available. E-mail your presentation to me no later than **August 8, 2005**. LCD projector guidelines are included in the enclosed Speaker Reference Guide.

Enclosed is an IAFP 2005 brochure. Additional meeting information is available on our Web site (www.foodprotection.org). Complete the enclosed registration form and return it along with payment to IAFP. Registration confirmation and other meeting details will be sent after we receive your registration. If you have further questions, contact me at 800.369.6337, 515.276.3344, or E-mail: bbrannen@foodprotection.org.

Sincerely,

Bev Brannen
Public Relations

Non-Presenter Policy: To ensure a successful IAFP Annual Meeting, it is essential for scheduled technical and poster presenters to appear at the time and date specified to give their presentation. Presenters who are unable to present should find a replacement presenter. If it is necessary to withdraw an abstract, IAFP must be notified in writing no later than 2 weeks prior to the meeting. Withdrawals made after the 2-week deadline will be subject to the non-presentation policy. Failing to present an accepted abstract at the scheduled time and place, or failure to find a replacement presenter, will result in the presenter being prohibited from presenting an abstract at any IAFP-sponsored meeting through the conclusion of the next year's Annual Meeting.

A COMPARATIVE STUDY AS RELATED TO THE EFFECTS OF GLUCOSE MONOHYDRATE, HOT WATER AND SODIUM PYROPHOSPHATE ON SOME QUALITY CRITERIA OF DEBONED AND MATURED BRISKET

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ABSTRACT

Organic acids, hot water, chlorine were commonly used in carcass decontamination for years. Organic acids were adversely effective on color and corrosive, while high temperatures of hot water was discoloring. Contrary, glucose fermentation by Lactic Acid Bacteria in meat during rigor, might be effective in microbial inhibition without adverse effect. Therefore, briskets were dipped in D(+) Glucose Monohydrate (GM) (16,51 g/100 ml, 15%) in the 7^{th}. day of maturation. Other primals were dipped in hot water(HW); Sodium Pyrophosphate (SPP) and HW; GM+SPP+HW; GM+HW, indicating GM+HW and GM+SPP+HW applications showed more microbial inhibition with lower acidity loss($p<0.05$ and $p<0.01$).

Key words: brisket, D(+)Glucose monohydrate, hot water, Sodium pyrophosphate (SPP), acidity, microbial quality.

INTRODUCTION

Decontamination of carcasses in the consideration of meat borne diseases, has been of interest for a number of years. Stivarius et al. (2002) treated beef trimmings in a tumbler with hot water at 82 °C and reduced *Escherichia coli, Coliform* and Mesophile Aerob Bacteria (MAB). Another application especially for poultry carcasses was hot water dip or spray, up to the 80 °C, although higher levels were discoloring. In some of these applications, sodium pyrophosphate (SPP) was intentionally added to increase thermal inactivation. Juneja and Marmer (1998) observed that addition of SPP as 0.15 % into ground beef during heat process had reduced D value of *Cl. perfringens*. Addition of SPP as 0.3% into the beef gravy in another study, increased heat sensitivity of *Listeria monocytogenes* contamination flora. However, water existence in these kind of applications, remaining on the carcass or in meat after washing, enhanced subsequent microbial growth. Organic acids have been other chemicals to decontaminate carcasses. Castillo et al. (2001) sprayed beef carcass surface with 4% lactic acid at 55 °C and observed significant reductions of *Escherichia coli* 0157:H7 while Samelis et al.(2001) inhibited *Listeria monocytogenes* by dipping sausages into the 5% lactic acid solution. But organic acids were also reported as discoloring and corrosive in the long run (Reuter, 1998).

Lactic Acid Bacteria (LAB), described as acid producers (Lero et al. 2002), were also used in meat decontamination. In one of these applications, spoilage of wheat bread and poultry waste were inhibited by *Lactobacillus* and *Pediococcus* (Katina et al. 2002; Jalil et al. 2001). Briefly, glucose fermentation by LAB, resulted with lactic acid

production during rigor, always became as one of the most responsible in inhibition of microbial growth. Any application which stimulates this fermentation such as LAB or glucose rather than organic acids, could be effective in enhancing of meat shelf life and decontamination, by increasing acidity without adverse effects mentioned above. Therefore, various concentrations of Glucose monohydrate (GM) solutions were treated with brisket sub-primals in the first part of this study as an alternative to decontaminate brisket meat cuts. In the last part, the dip of brisket into the most effective sugar concentration without causing any adverse effect on meat sensory, was compared with the hot water dip, hot water dip with SPP (0.3%), hot water then sugar solution dip, hot water dip with SPP then sugar solution dip and untreated meat as control. Finally, the most effective application in meat decontamination was determined and advised to improve quality of brisket sub-primals which can be applied followed by chilling & maturation as marked and shown in Fig. 1.

MATERIALS and METHODS

Sample preparation:

Beef cuts of brisket & fore shanks were obtained followed by maturation at fabrication stage. Maturation was applied for 2 days at 11 °C and then 5 °C for 4 days in 85% relative humidity (RH). Matured and cooled brisket & fore shanks, in the 7th day of maturation, were dipped into GM solutions in 3,5,7,9,10,13,15,17 and 21% concentrations, while one cut was kept as untreated as control in the first part of experimental design. These cuts were transferred to the +4 ◦C refrigeration condition and 300 grams from each application cut were taken into the sensory evaluation in the 4th day of shelf life at +4 ◦C. D(+)Glucose-monohydrate (GM) (Merck kGaA 64271 Darmstadt, Germany) solutions were prepared by melting GM (as 16,51g/100ml) at 80 °C and cooling back below 43 °C. The dip process of cuts into 15% GM solution among various sugar concentrations was found to be the most effective in improving microbial quality without any adverse effect on sensory criteria as indicated in Results & Discussion part. In the last part, other fore shank & brisket cuts, as treated with the same maturation & cooling process, were divided into six groups (as each has two cuts). Cuts in the first group were dipped in hot water at 95 °C for 10 sec (coded as; **HW**). SPP was dissolved in water at 80 °C and cooled below 43 °C. The cuts in second group were first immersed in this SPP(0.3%) solution for 15 min. then dipped in hot water (coded as;**SPP+HW**). Cuts of the third group were dipped in 15 % GM solution (which was found as most effective sugar solution) for 25 min. then dipped in SPP solution for 15 min., finally dipped in hot

water at 80 °C for 10 sec. (**GM+SPP+HW**). Cuts in fourth group were dipped in GM 15% for 15 min. then dipped in hot water for 10 sec. (coded as; **GM+HW**) while cuts of the fifth group were only dipped into GM 15% for 25 min. (coded as; **GM**). Finally the sixth and last group cuts were kept as untreated as control (coded as; **C**).

Microbial enumeration:

Cuts were transferred into the cooling units at 4 °C as in duplicate sterile plastic bags. Meat cuts as 0.5 g from surface and 0.5 g from 1 cm depth, were cut from each of two briskets in each group. These samples were ground and mixed homogeneously by using sanitized lancet and tweezers and 1 g from ground meat of each group was taken to the microbial analyses. Enumeration was carried using PCA (Oxoid) for Mesophile Aerob Bacteria (MAB), VRBA (Oxoid) for *Coliform*, Mac Concey Agar (Oxoid) for *Pseudomonas* and MRS (Oxoid) for Lactic Acid Bacteria (LAB). Pour plating and pH determinations were done as duplicates for each sample.

pH determination:

pH of the samples was measured after homogenization using a digital pH meter (Heidolph-Schott, Maintz). Microbial enumeration and pH determinations were made for 24h. after applications and then continued within 24 hours' intervals.

Sensory evaluation:

300 grams of each application group, dipped into various GM (3-21%) solutions, were taken into the ovens and cooked under 180 °C for 25 min. at the 4th. day of +4 °C shelf life. The cooked meats from each application, were evaluated as 'accepted' and 'rejected' by 15 panellists (randomly selected from different age, profession, sex and location) by using discrimination test (Meilgaard, Civille and Carr, 1991). The applications (different GM% solution applied groups) which were evaluated as accepted in whole sensory criteria by 9 panellists in this test, were selected. The selected ones in discrimination test, were then evaluated by descriptive test by other 15 panellists; previously trained as in three sessions about meat sensory for 2 weeks. Meats from each group as cooked under 180 °C for 25 min. were compared by these panellists from the point of tenderness, appearance, color, texture, aroma, odor and taste criteria by giving points between 1 (worst) and 10 (the best). All sensory assessments were performed under natural light. Natural spring water was provided between samples to cleanse the palate.

Statistical analysis:

Multivariate Statistical Analysis (Richard and Wichern, 1987) was applied to determine whether a variance is abundant among different GM% solution dip treated application groups from the point of sensory quality, whereas Univariate Analysis of

Variance and Dunnett test (Montgomery et al. 1997; Neter et al. 1999) were used to determine the variance among applications of the second part.

RESULTS and DISCUSSION

As mentioned in Material & Method, the fore shank & brisket cuts were dipped into 3,5,7,9,10,13,15,17 and 21% D(+)Glucose-monohydrate (GM) (Merck kGaA 64271 Darmstadt, Germany) solutions for 25 minutes. The cuts as mentioned in Material & Method, were cooked and evaluated by taste panel at the 4 th. day of shelf life under +4 °C. All application groups were accepted by taste panel in discrimination test and then the same application groups were compared by using descriptive test from the point of each quality criteria (tenderness, appearance, color, texture, aroma, odor and taste). According to the multivariate statistical analysis results, there was no difference among the application groups dipped into 3-15 GM% solutions in taste, odor and color criteria, whereas juiciness, tenderness and texture were adversely effected in 17-21%, compared with control. When LAB counts of application groups, dipped into 3-15 GM% solutions, are concerned, from the point of 'day' and 'application' parameters, variations were not equal and therefore Dunnett test was applied. The model of Dunnett test appears as $Y_{ijk}= \gamma + \alpha_i + \beta_i + (\alpha\beta)_{ij} + \Sigma ijk$ where γ: constant, α_i : main effect for factor 'day' at the concerned level, β_1 : mean effect for 'application' at 0,...,15%GM levels, $(\alpha\beta)_{ij}$: Interaction terms, Σijk : error terms. F test shows that 'day', 'application' and 'day & application' interaction appeared significantly important ($p<0.05$ and $p<0.01$) It can be clearly expressed as in the highlight of this statistical data (shown in Table 1) and LAB

counts of applications (Fig. 3) that LAB counts were increased by the increase of GM% in each day and all through 8 days of shelf life at +4 °C($p<0.05$ and $p<0.01$). On the other hand, the only explanation for the rejections of 17-21% GM applications by panellists in juiciness, texture and taste might be due to the higher concentration of GM with higher acidity and lower water binding capacity. The higher LAB counts than log 5.94 of 15%GM in 4^{th} day of shelf life(Fig 3) with its acidity, may have caused a decrease in water binding capacity, followed by, a tough texture with the pale color formation which may also appear as factors distracting panellist and causing them to reject %17-21 %GM application groups. Therefore only 15% GM solution treated group was selected as the most effective GM application group in increasing LAB without adversely effecting meat sensory.

In the second part, as previously mentioned, matured and cold fore shank & brisket cuts were dipped into 15 % GM (**GM**) as the first application group. Cuts in the second group were dipped in hot water (**HW**) while the third group of fore shank & brisket cuts were first dipped in Sodium Pyrophosphate (SPP)(0.03%) solution and then dipped in hot water(**SPP+HW**). Cuts of the fourth group were dipped in 15% GM solution, then dipped in SPP (0.3%) and finally dipped in hot water (**GM+SPP+HW**). Cuts in the fifth group were dipped in 15%GM solution and then dipped in hot water (**GM+HW**), while the last group was kept as untreated as control (**C**). SPP, GM, HW preparations with the incubation times for dipping in these solutions were indicated in 'Material & Method' part. When all these different protective application groups were compared from the point of pH, because of unequal variations, Dunnett test was applied.

According to the Dunnett test results, all applications appeared as significantly different. Also the difference among applications in each day of 9 days of shelf life at +4 °C and the effect of shelf life on pH appeared as significantly important ($p<0.05$ and $p<0.01$). The pH levels belong to applications, increased through the shelf life indicating an acidity loss by time. The pH values belong to GM+HW were found as the lowest, indicating highest acidity all through the shelf life, followed by GM+SPP+HW, HW and GM applications orderly, while the lowest acidity was detected in Control ($p<0.05$ and $p<0.01$). (Table 2 and Fig. 6).

In the consideration of Mesophile Aerob Bacteria (MAB) counts of the concerned protective applications, according to the Dunnett test results, there is significant difference among applications in each day of shelf life and all through the shelf life ($p<0.05$ and $p<0.01$). The lowest MAB counts were detected in GM+HW followed by GM+SPP+HW, HW and GM applications orderly, while the highest MAB growth was observed in control (Table 3 and Fig.2). The concerned MAB inhibition by synergistic effect of GM, SPP with HW also GM and HW combined applications were also confirmed by the lowest acidity losses of the same applications, while SPP's increasing effect of heat penetration as an expected pasteurization enhancer, did not appear as effective as these two mentioned applications and also others except control.

Pseudomonas spp., as the spoilage indicators in refrigeration conditions, were most inhibited by GM+HW and GM+SPP+HW in each day of shelf life under + 4 °C. SPP's increasing effect of heat penetration in pasteurization SPP+HW, again did not

become as effective as GM+HW, GM+SPP+HW, HW and GM, except control ($p<0.05$ and $p<0.01$). GM's acid and LAB count increasing effect with the heat of HW, in addition to SPP which eases the heat penetration, may be the main inhibitory effects of these concerned applications on *Pseudomonas* (Table 4 and Fig. 4).

The same effects on *Pseudomonas* spp. of the same applications mentioned above, were detected on *Coliform*, indicating as GM+HW and GM+SPP+HW are the most inhibitory ($p<0.05$ and $p<0.01$). SPP's increasing effect of heat penetration in pasteurization SPP+HW, was not found effective on *Coliform*, similar to *Pseudomonas* spp., in comparison with the other applications except control (Table 5 and Fig 5). All *Pseudomonas* and *Coliform* counts, like MAB, increased by time through the shelf life under +4 ∘C with acidity losses ($p<0.05$ and $p<0.01$).

When the microbial values in 8th. day are concerned, in this last period of shelf life, 1,63 and 1, 41 log reductions in *Coliform*, made by GM+HW and GM+SPP+HW; 1,90 and 1,80 log reductions in *Pseudomonas* spp., made by again GM+HW and GM+SPP+HW orderly, indicate significant protective effect of the concerned applications. log 0.83 and 0,72 MAB reductions in MAB of GM+SPP+HW and GM+HW applications with their less acid losses, confirm this protective effect of the same applications on beef fore shank-brisket primal cuts. GM's synergistic and protective effect with HW and SPP may be due to it's stimulation on LAB, while decreasing acidity loss during shelf life. Unfortunately, there is not enough study as related to the protective effect of glucose or GM in meat industry, while highlighting the interaction between

LAB and meat pathogens like *Salmonella*. According to the results of this study mentioned above, the dip of beef primal cuts into GM(15%) solution, could be used as a protective application after stunning, skinned-off and washing process step of carcasses as alone, or combined with HW and also SPP and HW applications, without any adverse effect on meat sensory.

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instrumental color and sensory properties of ground beef during display. Meat Science. 60(4):327-334.

FIGURE LEGENDS

Figure 1. Beef slaughter flow diagram, which indicates the application point of GM, GM+HW, GM+SPP+HW, HW and SPP+HW experimental procedures as marked, in microbial improvement of sub primals.

Figure 2. MAB counts of control and application groups.

Figure 3. LAB counts of cuts which were dipped in 3-15% D(+) GM solutions.

Figure 4. *Pseudomonas* spp. counts of control and application groups.

Figure 5. *Coliform* counts of control and application groups.

Figure 6. pH levels which belong to control and application primal cuts.

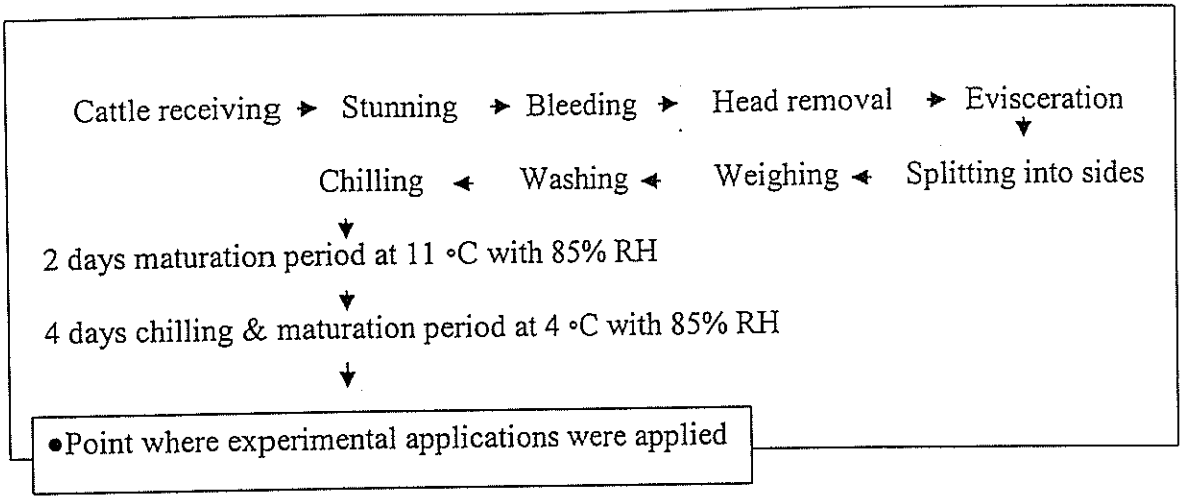

Cattle receiving
Stunning
Bleeding
Head removal
Evisceration
Splitting into sides
Weighing
Washing
Chilling
2 days maturation period at 11 °C with 85% RH
4 days chilling & maturation period at 4 °C with 85% RH
•Point where experimental applications were applied

Fig. 1

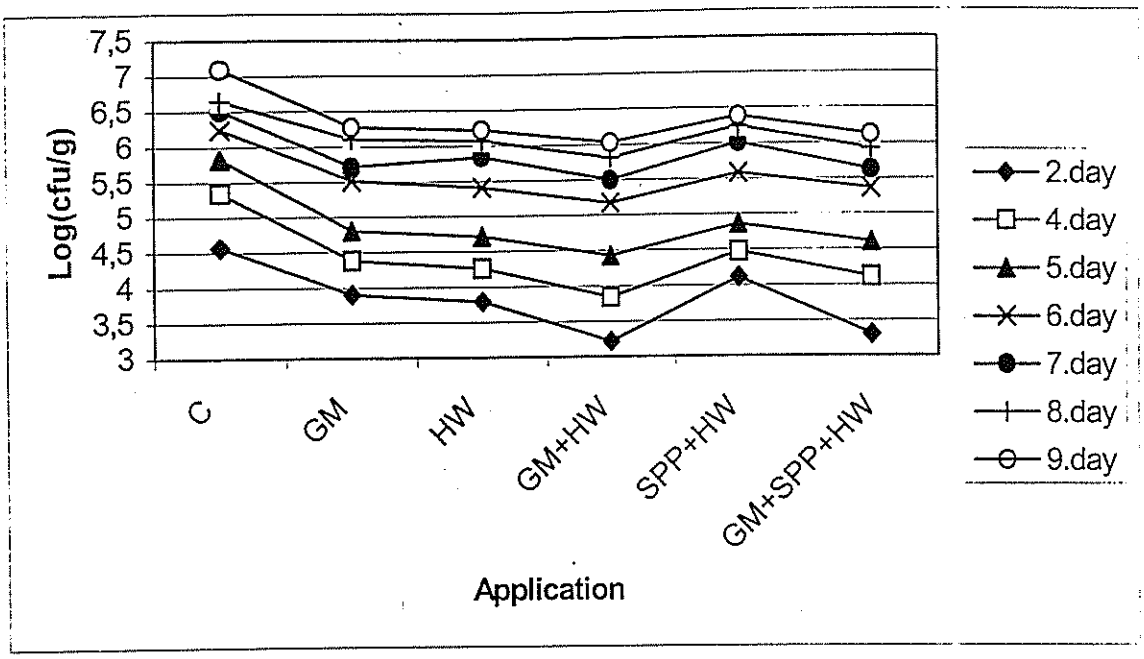


Fig. 2. MAB counts of control and application groups.

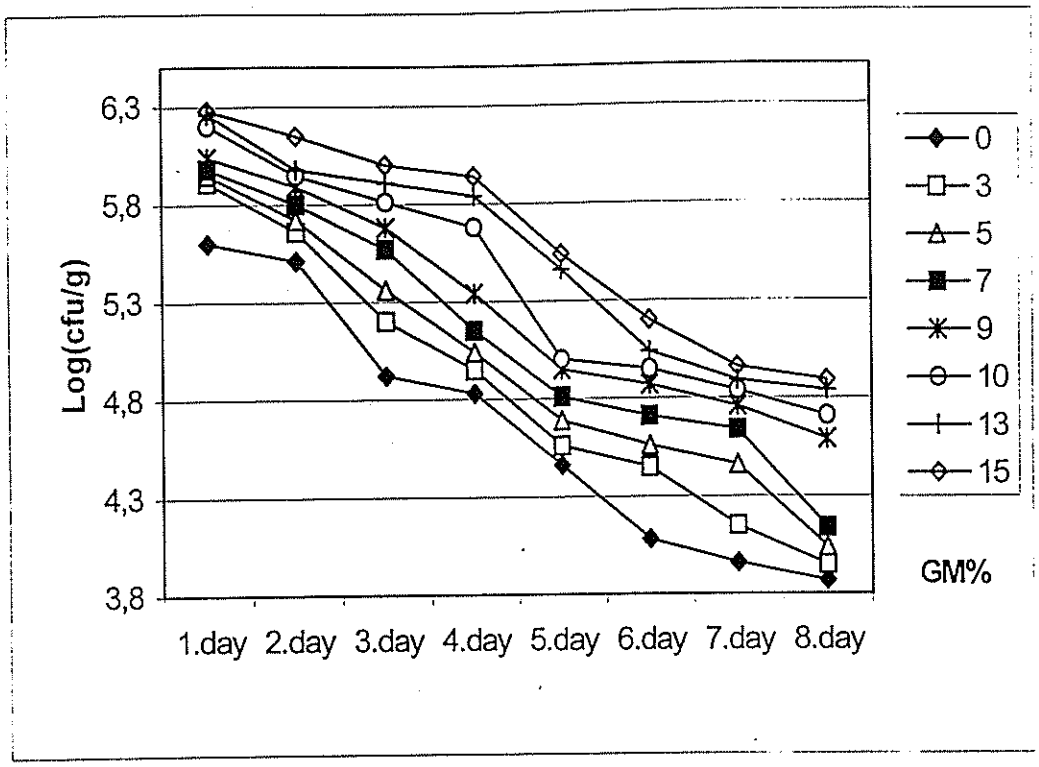


Fig. 3. LAB counts of cuts which were dipped in 3-15% D(+) GM solutions.

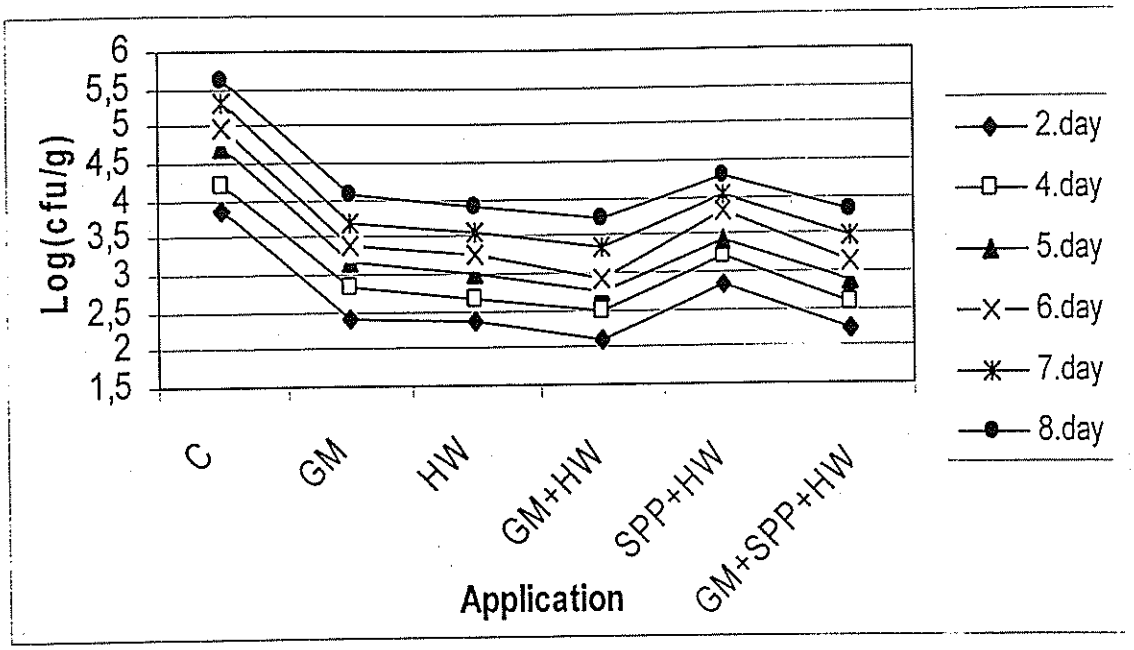


Fig. 4. *Pseudomonas* spp. counts of control and application groups.

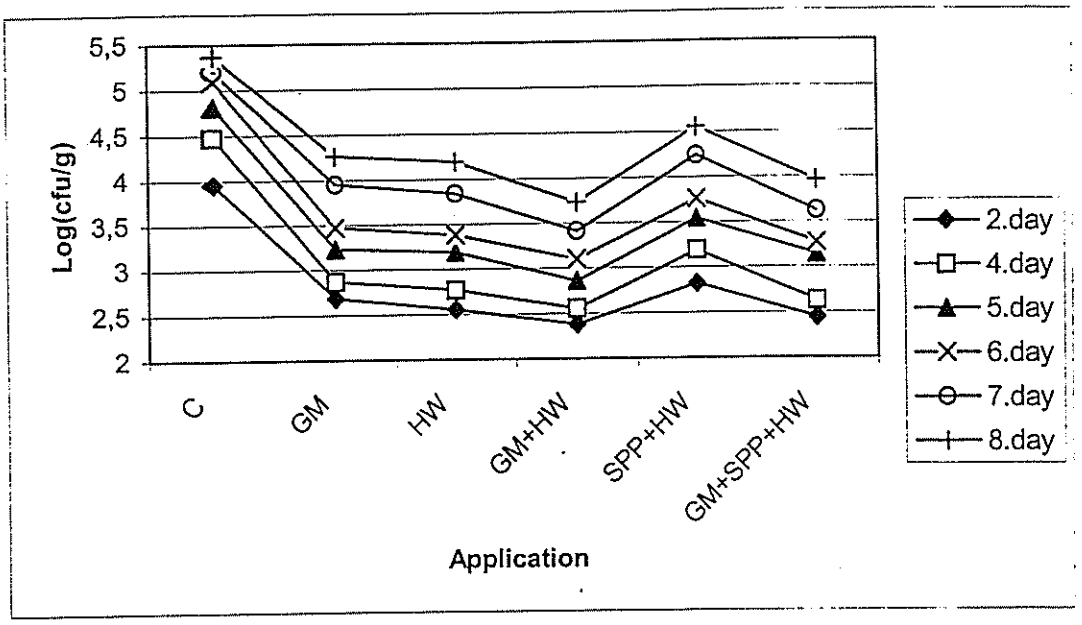


Fig. 5. *Coliform* counts of control and application groups.

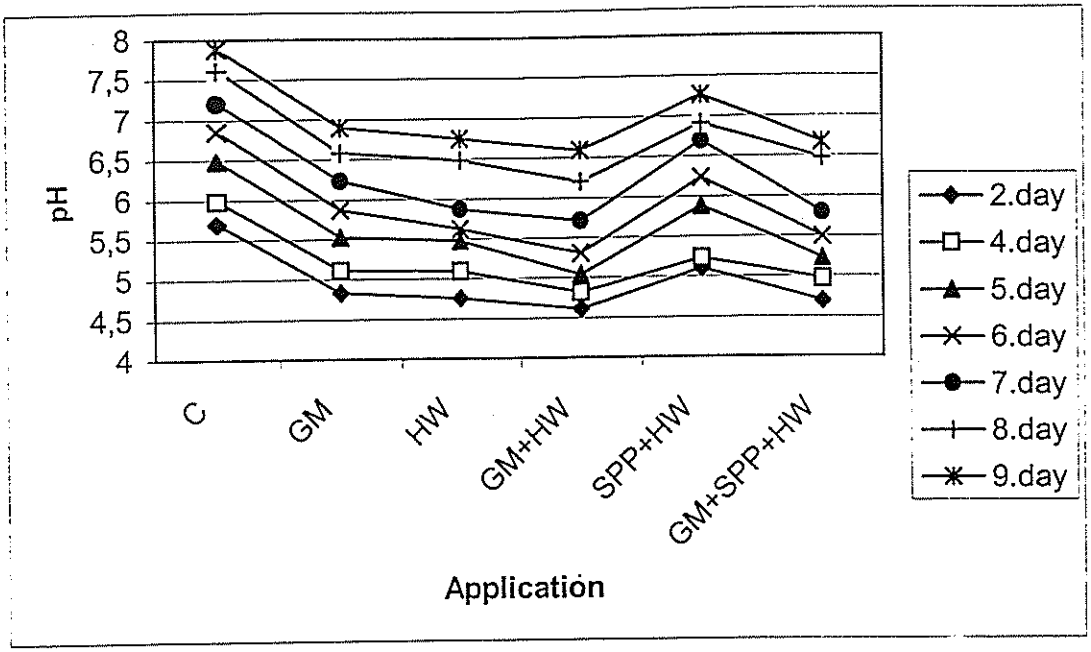


Fig. 6. pH levels which belong to the primal cuts of control and applications.

TABLES

Table 1. Variations, F test results of Dunnett test indicating the importance of %3-15 GM solution dip on LAB counts of fore shank & brisket in each day and through 8 days of shelf life under +4 °C, %85 RH cooling conditions.

Table 2. Variations, F test and Dunnett test results with mean values and standard errors of pH belong to applications in each day and all through the shelf life.

Table 3. Variations, F test and Dunnett test results with mean values and standard errors of MAB belong to applications in each day and all through the shelf life.

Table 4. Variations, F test and Dunnett test results with mean values and standard errors of *Pseudomonas* spp. belong to applications in each day and all through the shelf life.

Table 5. Variations, F test and Dunnett test results with mean values and standard errors of *Coliform* belong to applications in each day and all through the shelf life.

Source	Type III Sum of Squares	df	Mean square	F	Sig.
Corrected model	55,464[a]	63	,880	802,626	,000
Intercept	3427,299	1	3427,299	3124603,1	,000
Day	41,361	7	5,909	5386,910	,000
Application	12,769	7	1,824	1663,081	,000
Day & Application	1,333	49	2,721E-02	24,806	,000
Error	7,020E-02	64	1,097E-03		
Total	3482,833	128			
Corrected Total	55,534	127			

a. R Squared= ,999(Adjusted R Squared= ,999)

Table 1. Variation and F test results indicating the importance of %3-15 GM solution dip on LAB counts of fore shank & brisket in each day and through 8 days of shelf life under +4 °C, %85 RH.

Source	Type III Sum of Squares	df	Mean square	F	Sig.
Corrected model	59,667[a]	41	1,455	2186, 827	,000
Intercept	2971, 549	1	2971,549	4465297,9	,000
Day	42,361	6	7,060	10609,081	,000
Application	16,567	5	3,313	4979,031	,000
Day & Application	0,739	30	2,463E-02	37,008	,000
Error	2,795E-02	42	6,655E-04		
Total	3031,244	84			
Corrected Total	59,694	83			

a. R Squared= 1,000(Adjusted R Squared= ,999)

Application → / Day;	C	HW	GM	SPP+HW	GM+SPP+HW	GM+HW
2	5,69*	4,75	4,83	5,10	4,69	4,60
	2,828E-02**	4,243E-02	2,828E-02	4,243E-02	4,243E-02	7,071E-03
4	5,97	5,09	5,11	5,23	4,97	4,82
	7,071E-03	2,828E-02	,0000	2,828E-02	,0000	2,828E-02
5	6,48	5,46	5,53	5,87	5,22	5,05
	4,243E-02	7,071E-03	2,828E-02	7,071E-03	2,828E-02	5,657E-02
6	6,84	5,62	5,86	6,23	5,49	5,31
	7,071E-03	2,828E-03	7,071E-03	2,828E-02	4,243E-02	,0000
7	7,19	5,87	6,23	6,68	5,78	5,71
	7,071E-03	1,414E-02	4,243E-02	7,071E-03	7,071E-03	2,828E-02
8	7,61	6,48	6,57	6,91	6,67	6,20
	2,828E-02	2,828E-02	7,071E-03	1,414E-02	,0000	,0000
9	7,88	6,74	6,90	7,26	6,68	6,59
	4,243E-02	7,071E-03	,0000	,0000	2,828E-02	2,828E-02

Table 2. Variations, F test and Dunnett test results with mean values and standard errors of pH belong to applications in each day and all through the shelf life.

*: mean value **: Standard error

Source	Type III Sum of Squares	df	Mean square	F	Sig.
Corrected model	77,373[a]	41	1,887	13209,987	,000
Intercept	2342,208	1	2342,208	16395456	,000
Day	64,741	6	10,790	75531,339	,000
Application	11,812	5	2,362	16536,373	,000
Day&Application	0,820	30	2,733E-02	191,319	,000
Error	6,000E-03	42	1,429E-04		
Total	2419,587	84			
Corrected Total	77,379	83			

b. R Squared= 1,000(Adjusted R Squared= ,999)

Application → / Day;	C	HW	GM	SPP+HW	GM+SPP+HW	GM+HW
2	4,56	3,79	3,90	4,10	3,21	3,29
	,0000	,0000	,0000	7,071E-03	1,414E-02	7,071E-03
4	5,34	4,26	4,37	4,46	3,83	4,11
	1,414E-02	1,41E-02	7,071E-03	7,071E-03	7,071E-02	1,414E-02
5	5,82	4,71	4,80	4,86	4,41	4,59
	2,828E-02	1,414E-02	,0000	1,414E-02	7,071E-03	7,071E-03
6	6,23	5,41	5,51	5,57	5,16	5,36
	7,071E-03	1,414E-02	1,414E-02	7,071E-02	7,071E-03	1,414E-02
7	6,51	5,83	5,71	6,01	5,49	5,62
	,0000	7,071E-03	1,414E-02	7,071E-03	7,071E-03	2,828E-02
8	6,64	6,07	6,10	6,23	5,81	5,92
	7,071E-03	,0000	,0000	7,071E-03	1,414E-02	1,414E-02
9	7,10	6,22	6,27	6,39	6,02	6,12
	,0000	2,828E-02	1,414E-02	1,414E-02	7,071E-03	,0000

Table 3. . Variations, F test and Dunnett test results with mean values and standard errors of MAB belong to applications in each day and all through the shelf life.

Source	Type III Sum of Squares	df	Mean square	F	Sig.
Corrected model	50,205[a]	35	1,434	25189,973	,000
Intercept	852,914	1	852,914	14977999	,000
Day	20,726	5	4,145	72793,956	,000
Application	29,280	5	5,856	102837,80	,000
Day & Application	0,820	30	2,733E-02	191,319	,000
Error	2,050E-03	36	5,694E-05		
Total	903,121	72			
Corrected Total	50,207	71			

c. R Squared= 1,000(Adjusted R Squared= 1,000)

Application → / Day;	C	HW	GM	SPP+HW	GM+SPP+HW	GM+HW
2	3,87	2,35	2,42	2,84	2,21	2,10
	,0000	7,071E-03	1,414E-02	1,414E-02	7,071E-03	,0001
4	4,18	2,64	2,85	3,20	2,56	2,48
	7,071E-03	,000	,000	7,071E-03	7,071E-03	7,071E-02
5	4,73	3,01	3,18	3,43	2,85	2,74
	1,414E-02	,0000	7,071E-03	7,071E-03	7,071E-03	,000
6	4,95	3,24	3,38	3,80	3,11	2,92
	7,071E-03	7,071E-03	,000	7,071E-03	,000	1,414E-02
7	5,30	3,56	3,69	4,04	3,47	3,35
	,0000	7,071E-03	1,414E-02	,000	1,414E-02	7,071E-03
8	5,62	3,90	4,05	4,26	3,82	3,72
	,0000	7,071E-03	7,071E-03	7,071E-03	7,071E-03	,000
9	4,77	3,12	3,26	3,59	3,00	2,88
	,6312	,5490	,5577	,5115	,5637	,5587

Table 4. Variations, F test and Dunnett test results with mean values and standard errors of *Pseudomonas* spp. belong to applications in each day and all through the shelf life.

Source	Type III Sum of Squares	df	Mean square	F	Sig.
Corrected model	46,000[a]	35	1,314	9755,540	,000
Intercept	915,563	1	915,563	6795934,3	,000
Day	20,048	5	4,010	29761,569	,000
Application	25,484	5	5,097	37831,928	,000
Day&Application	0,468	25	1,873E-02	139,057	,000
Error	4,850E-03	36	1,347E-04		
Total	961,568	72			
Corrected Total	46,005	71			

d. R Squared= 1,000(Adjusted R Squared= 1,000)

Application → / Day;	C	HW	GM	SPP+HW	GM+SPP+HW	GM+HW
2	3,94	2,55	2,68	2,82	2,43	2,37
	7,071E-03	,0000	1,414E-02	,0000	7,071E-03	7,071E-03
4	4,47	2,76	2,87	3,19	2,62	2,55
	1,414E-02	7,071E-03	1,414E-02	1,414E-02	7,071E-03	,000
5	4,81	3,19	3,22	3,53	3,14	2,86
	1,414E-02	,0000	7,071E-03	7,071E-03	,0000	1,414E-02
6	5,09	3,37	3,47	3,75	3,26	3,10
	,0000	7,071E-03	1,414E-02	7,071E-03	7,071E-03	,000
7	5,19	3,84	3,94	4,24	3,62	3,41
	7,071E-03	2,828E-02	7,071E-03	,000	2,828E-02	1,414E-02
8	5,36	4,18	4,25	4,54	3,95	3,73
	7,071E-03	7,071E-03	7,071E-03	7,071E-03	7,071E-03	2,828E-02

Table 5. Variations, F test and Dunnett test results with mean values and standard errors of *Coliform* belong to applications in each day and all through the shelf life.