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DESCRIPTION OF MICROBIAL AND CHEMICAL CHANGES DURING THE TENDERIZATION PROCESS OF BUFFALO MEAT

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ABSTRACT

The microbiological examinations of tested meat and curing solution were carried out using different cultivation media. Screening programm of obtained isolates was performed on milk agar medium to detect their potentialities against milk casein. Chemical description was also followed together with determination of sugars values in the curing solution during the whole process. Results obtained revealed that both meat and immersing solution were different from each other from microbiological and chemical point of view. Positive linear regression was obtained by plotting values of H/G% either against growth diameter or against hydrolysis zones. Profiles of both pH and optical density of the curing solution seems to be constant during the whole process. Results of the total protein exhibited gradual increase up to the 9th day of the process. Values of non reducing sugars and total sugars showed gradual decrease up to 6th day then increased again while reducing sugars showed a steady state during the treatment period.

INTRODUCTION

Most of the preservative effect of the curing agents, is attributed to the sodium chloride, with some bacteriostatic effect from other components added. The salt, sugar, and meat protein combine to lower the a_w value of the cured meats, e.g., of hams to about 0.95 to 0.97. Other preservative factor is the low curing temperature. Throughout the world, particularly in developing countries, the consumption of food contaminated by poisoning bacteria cause a serious public health problems. This problem continue to affect millions of the world population while no real solution is in sight. Though the curing process is one of the methods used to inhibit or inactivate the growth of contamining pathogenic bacteria in meat. This method is of paramount importance because it lead also to the tenderization of meat beside the purpose mentioned above. Also, proteolytic enzymes were applied to the meat industry as meat tenderizers. The action of proteases on meat tenderness depends on many factors i.e., optimum pH, enzyme concentration, ability of the enzyme to penetrate meat, enzyme stability at cooking temperatures. So, the action of proteases on meat characteristics under the optimum conditions would improve its tenderness, quality and digestibility as well (Frazier and Westhoff, 1978).

Dainty *et al.* (1985) found that microflora present in meat is very diverse, although it is mainly mesophiles, i.e. *Micrococci, Staphylococci*, *Bacillus sp, Coryneforms, Enterobacteria, Flavobacteria, Pseudomonas*, and lactic acid bacteria. These organisms are distributed on lean, fat and connective tissues each of which can support the microbial growth. Lean tissues provide a rich variety of readily available sources of energy, carbon

and other nutrients at pH values highly conducive to growth, i.e. typically in the range pH 5.5 - 6.5. Induced depletion of glycogen in the live animal that leads, in turn, to low concentration of glucose and lactic acid in the post mortem of meat. This combination of high pH and low glucose has important consequences for tenderization processes under certain conditions. Dainty and Mackey (1992) found that after dressing, red meat typically carry between 10^2 - 10^4 bacteria/cm^2. These bacteria almost derived from hide, faces, jut contents, hands and instruments of slaughter-persons.

The main non-nitrogenous compounds in meat mostly used to obtain energy for microorganisms and possibly serving as sources of carbon, include carbohydrates and organic acids. Complex di-tri- or polysaccharides are hydrolyzed to simple sugars before utilization. Organic acids are oxidized by microbes to carbonates which causing the medium to become more alkaline. Anjaneyulu *et al*., (1990) examined the effect of salt on buffalo meat and its effect on the chemical properties during refrigerated storage. The aim of this investigation is to trace some of both microbial and chemical changes occurred in the curing solution during the whole period of the pickling process.

MATERIALS AND METHODS

I- Materials:

I-1. Examined meat samples:

Buffalo meat samples examined in this investigation were collected from local market, Kafr El-Sheikh City, Kafr El-Sheikh, Egypt.

I-2. Curing solution :

The tenderization of meat in study was carried out in a curing solution using meat weight of 1.324 Kg. The composition of curing solution is shown in Table (1).

Table (1): Ingredient of the curing solution.

Components	Wt./g
Ginger (*Zingiber officinale)*	5.0
Coriander (*Coriander sativum)*	5.0
Garlic (*Allium sativum)*	10.0
Black pepper (*Piper nigrum)*	5.0
Red pepper (*Capasicum annum)*	5.0
Glucose	150.0
Sodium chloride	450.0
Water	3500 ml

All the components were put in water (3.5 L), then boiled for 10 min. After which, the mixture was filtered and in the obtained filterate the tested meat was put. The container was put in the refrigerator at 5°C for 10 days experimental period. During this period, samples were taken every day for analysis.

I-3. Cultivation media:

The following general and specific cultivation media were used for different purposes according to the Bacteriological Analytical Manual for

Foods (FDA, 1976). Tryptone glucose agar (TGY), was used for isolation and for total count of the microbial load of the curing solution at 7.0 - 7.2.; Milk agar (MA), was used for isolation, counting and screening of proteolytic bacteria at pH 7.0 - 7.2; Fuchsin lactose agar (Endo C agar), was used to detect the bacterial members of the family *Enterobacteriaceae*. Mannitol sodium chloride phenol red agar (MSC), was specifically used for detection of *Staphylococcus spp*. Sabouraud glucose agar (SGA), was used for the detection of fungi and yeast. Membrane-filtration Enterococcus selective agar medium (MFE) was selectively used for *Enterococcus*. Bacto agar F - medium (BAF) is recommended for the detection of *Pseudomonas* spp. Nutrient agar containing oil was used for isolation and counting the lipolytic bacteria. The composition of these culture media was as described in Oxoid, (1982).

II- Methods :

II-1. Bacteriological procedures :

II-1-1. Isolation of microorganisms naturally occurred in curing-solution:

One ml sample of examined curing solution after the 5th day at low temperature, was dispersed in appropriate volume (9 ml) of distilled water and different serial dilutions were made with vigorous shaking to give a final dilution of 10^{-3}. One ml sample was then taken and plated on different cultivation media and appropriate periods of incubation were used according to the purpose required as follow:

a) Plates of MA were flooded with 10 ml of 15 g $HgCl_2$ solution in 100 ml distilled water and 20 ml of concentrated HCl (Smith *et al.*, 1952). The presence of a clear zone around the colonies against an opaque white background indicated that the organisms were proteolytic. The diameter of each clear zone was measured as a rough indication of proteolytic activity of the organisms. The organisms which showed considerably large zones of protein solubilization were selected for further studies, (Srinivasan *et al.*, 1964).
b) Lipolytic bacteria were determined on NA containing 1% olive oil, after appropriate period of incubation at 37°C and 7°C, plates were flooded with concentrated $CuSO_4$ solution. Bluish green colonies surrounded by precipitates were detected as lipolytic bacteria (Mourey and Kilbertus, 1976).
c) Other bacterial genera were detected and counted on other specific media noted above (media used).
d) The fermentation of mannitol to acid, which serves as a guideline for pathogenicity, is indicated by a colour change of the pH indicator, phenol red, (Standard Methods for the Examination of Water and Wastewater, 1992).
e) Total bacterial count was carried out using TGY medium.

II-1-2. Purification of obtained isolates :

From the preceding step, well separated colonies were selected and transferred to the slope agar. In order to make sure of the purity of these

isolates, each of them was suspended in sterile water and recultivated again for two cycles followed by single colony isolation after streaking onto plates. Slope cultures were considered pure when they showed uniform morphological feature by microscopic examination.

II-1-3. Maintenance of selected strains :

Selected bacterial strains were all maintained on NA slant at 5°C till use. Prior to use, the microbial cultures were transferred to NA and reincubated again at appropriate temperature for 48 hr. This process was repeated twice.

II-1-4. Screening method for qualitative proteinase assay :

The method used for qualitative proteinase assay was similar to that used in testing the antibiotics by diffusion method according to Collins and Lyne, (1985). The activity of the proteinase was indicated by measuring the clear zones (mm) surrounding the microbial colonies.

II-2. Chemical Analysis :

II-2-1. Sugar determination :

It was done according to the method of Nelson (1944) as modified by Somogyi (1952). In this method, 1 ml of the copper reagent was added to 1 ml of the test solution, mixed and placed in a boiling water bath for 10 min. After cooling, 1 ml of the arsenomolybdate reagent was added, mixed and the tubes left to stand at room temperature for 20 min. The samples were then diluted to 10 ml with distilled water. The absorbance of the samples were spectrophotometerically measured together with water and reagent blanks at 500 nm wavelength using a PYE Unicam Ltd SP-400 UV spectrophotometer. The amount of reducing sugar present was estimated from calibration curve using glucose standard solution.

II-2-2. Determination of protein :

The total protein content of the meat samples was measured by the micro-kjeldahl procedure after excluding the non-protein nitrogen as described by Word and Johnson (1962).

II-2-3. Measurement of pH :

Ten gm of tested sample to be extracted by 100 ml of distilled water using a blender for 5 min. After standing 30 min at room temperature, the sample was filtered. The pH of the obtained solution was measured using a pH-meter (CG 710 Schott-Gmbtt, Germany).

RESULTS AND DISCUSSION

I- Microbiological Examinations of the Tested Meat and Curing Solution:

Since meat is protein in nature and is capable of supplying consumers with nutrients, it is equally capable of supporting the growth of contaminating microorganisms. Eight culture media either general, or specific, were applied for the microbiological examination of tested meat and curing solution. The curing process was carried out at 5°C for ten days. The curing temperature, especially with a curing solution, usually is about 2.2 to 3.3°C. The time of the

cure varies with the methods used and the meats to be cured. The older methods of curing in the pickle require several months. The newer quick cure in which the meat are immersed in a curing solution of the ingredients shortens that time (Frazier and Westhoff, 1978).

This can benifit to protect from some zoonotic diseases of food borne infection, since a wide range of infection agents are ingested through food and/or water causing several syndromes. Gastro-enteritis as manifested by diarrhea and vomition results from infection with *Campylobacter sp*. from cows and chickens. Moreover, bacterial food poisoning causing intestinal disturbances and other syndromes results from infection with *Salmonella sp* and *Clostridium sp* from several animals, (Frazier and Westhoff, 1978).

The preliminary examination of the tested meat indicated the presence of different types of microorganisms as a result of slaughtering process and handling as well. These halophilic and halotolerant microorganisms represent the contamination from different factors. The boiling treatment of the curing solution at 100°C in the first preparation succeeded to overcome most of these microorganisms. This process is also likely to eliminate non spore-forming pathogens present in modest numbers as well as the vegetative cells of spore formers.

When using Endo C culture medium, sample of curing solution showed no growth at the first day (control), which was taken after boiling in the curing solution. After the 10th day, the number of *Enterobacteriaceae* members reached 3.5 x 10^3 cfu as shown in Table (2). On the other hand, the meat sample recorded 6.0 x 10^3 cfu at the first day but this number decreased to only about 0.37 fold after the period of the tenderization process (10 days). Furthermore, the number of *Enterobacteriaceae* members represent about 0.4 of the total viable count using TGY medium in case of curing solution after the tenth day. For meat, this value was 0.17 in case of the control while it was 0.04 after the 10th day. This mean that the curing process at 5°C decrease the bacterial count of family *Enterobacteriaceae* by 5.0 times in the meat samples during the whole process. So, this curing process is of great importance in overcoming the most bacterial species at low temperature (10°C) belonged to *Enterobacteriaceae* (Gill and Newton, 1977).

On milk agar medium (MA), examined curing solution gave 4.9 fold of proteolytic bacteria after the 10th day higher than that of the control (1st day). Meat sample, on the other hand, showed about 4.4 change fold for the treated sample compared to its control (Table 2).

In addition, the proteolytic bacteria isolated on MA medium represent about 0.3 of the total count value when using TGY medium at the beginning of the process while it reached to 0.6 after the 10th day for the curing solution. This mean that the value of change fold increased two times higher after 10 days than that of control when using TGY as culture medium. Concerning the meat sample, data showed that the proteolytic bacteria represent about 0.08 in the first day of the treatment while it reached to 0.2 after the 10th day of the process. This proved that the proteolytic bacteria were developed to about 2.5 times during the process (Table 2). This can also suggest their role in meat tenderness (El-Fadaly *et al*., 1998a and 1998b).

Table (2): Microbiological examination of the tested meat and the curing solution.

Investigated material	Examination time (day)	Total viable count, (cfu) x 10^3 **								Represent ratio of ***			
		TGY	MSC	Endo C	MFE	MA	SGA	OA	BAF*	Endo C	MA	OA	BAF
	1.0 (Control)	4.2	-	-	-	1.2	-	-	2.8	-	0.29	-	0.67
Curing solution	10.0 (treatment)	9.3	-	3.5	-	5.9	-	1.2	6.0	0.39	0.63	0.13	0.65
	Change fold	2.2	-	-	-	4.9	-	-	2.1	-	2.03	-	0.95
	1.0 (Control)	35	-	6.0	-	2.8	-	1.2	2.1	0.20	0.08	0.03	0.06
Meat	10.0 (treatment)	56	-	2.4	-	12.2	-	1.6	5.1	0.04	0.20	0.03	0.10
	Change fold	1.6	-	0.4	-	4.4	-	1.3	2.4	5.00	2.50	1.00	1.70

*** See FDA, (1976).**
**** cfu/ml of curing solution, cfu/gm of tested meat**
***** Calculated on the basis to the totla count (TGY).**

Using nutrient agar medium containg oil (OA), gave negative results in case of control while 1.2 x 10^3 cfu/ml of the curing solution were counted after 10 days. Besides, 1.3 change fold was found higher for treated meat than its control. On the other hand, the examined curing solution showed 0.1 change fold compared to the value when using TGY medium after the period of the process. It means that curing solution do not permit the microbial growth so well as for lipolytic bacteria. For meat, the lipolytic bacteria exhibited 0.03 change fold for both control and after 10 days of the treatment. These results suggest that the change fold remain constant during the whole period of the curing treatment. Sporeforming bacteria represented the most predominant bacterial types occurred in the examined samples using either MA medium or NA containg oil beside short rod bacteria (Table 2).

For detection of *Pseudomonas sp*, using of Bacto-agar F-medium showed 2.1 change fold for curing solution after 10 days treatment over its first day. Obtained results also exhibited 2.4 change fold of treated meat compared to the control. Nortije *et al*., (1990) isolated *Pseudomonas spp* and *Acinetobacter spp* and other members of *Enterobacteriaceae* from meat. The value of *Pseudomonas spp* was found to be 0.7 compared to the total count of the first day for the curing solution and this value remain constant during the whole process. In case of meat, *Pseudomonas spp* represent 0.06 of the total count measured at the first day. After the 10th day, the value became 0.1 which proved that the development of this type of bacteria developed to 0.6 during ten days which emphasizes the effect of curing process. Manganelli *et al*. (1993) found that *Enterobacteriaceae* and *Pseudomonadaceae* were the most frequently encountered bacteria. They also found that the most frequent *Enterobacteria* were *Escherichia coli, Enterobacter agglormerans, Serratia liquefaciens* and *Citrobacter freundii*.

It can be mentioned that the presence of these types of micro-organisms explain the relative high incidence of spoilage of meat, therefore, much work has been focused on the prevention of spoilage of meat products by *Pseudomonas sp* (Erichsen and Molin, 1981). Meanwhile, in order to examine for pathogenic bacteria, both of the curing solution and meat used in

this work exhibited negative results with the specific cultivation media for *Staphylococcus sp, Enterococcus sp,* as well as yeast and fungi. These results were noticed either in the first day (control) or after the whole period of treatment (10 days). These results suggested that meat samples were free of any disease. Prieto *et al*., (1995) isolated more than two hundred isolates of family *Micrococcaceae* from stored meat of which *Staphylococcus spp* represent their majority. The finding by Kirov *et al*., (1990) that some enterotoxigenic strains of *Aeromonas* may also be psychrotrophic and grow at temperatures of 4 to 5°C under varying salt concentrations and pH values which increase the possibility that food may be a mean of infection with these organisms.

II- Study of proteolytic activity of some bacterial isolates:

In order to detect the enzymatic activity of obtained isolates (short rods, sporeformers and coccoid-shaped bacteria), the examination was first conducted on milk agar plates (11.5 cm in diameter). Results demonstrate the reaction of the tested isolates expressed in the diameters of clear zones around the positive bacterial colonies which obtained from the hydrolysis of milk casein as shown in Fig. (1).

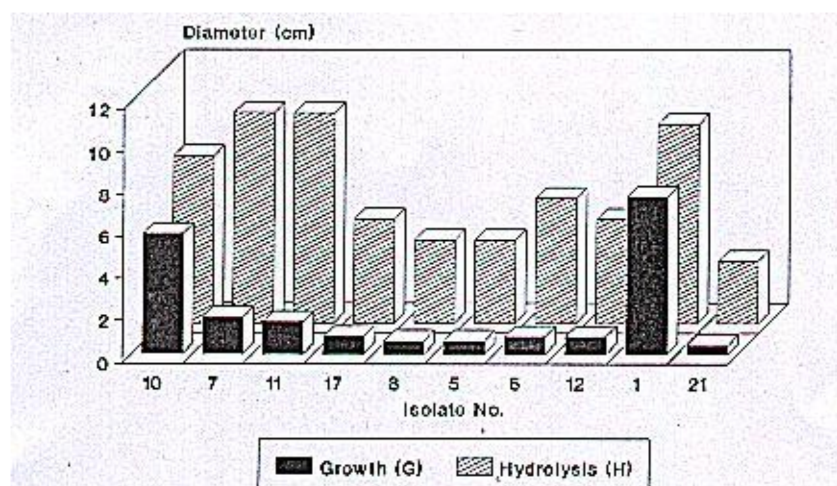

Fig. (1): Differences between growth and hydrolysis zone of bacterial isolates of the curing solution.

Results of the bacterial growth (G) showed that isolate No. 1 (short-rod) is the most active isolate in its growth followed by isolate No. 10 (spore former) being 5.7 cm growth diameter . Then the isolate No. 7 that give 1.7 cm growth diameter while isolate No. 11 comes later being 1.5 cm growth diameter and both of them are sporeformers. The potentialities of these isolates in casein hydrolysis is an indicator to their activities against the protein of meat. The zone diameters of hydrolysis (H) were found to be 10.0 cm for the isolates No. 7 & 11 followed by isolate No. 1 being 9.5 cm while the isolate No. 10 showed 8.0 cm clear zone (Fig. 1).

Even so, the evaluation of these isolates was made on the basis of the value of H/G %. Obtained results are graphed in Fig. (2). The most powerful was isolate No. 7 followed by isolate No. 6, then isolate No. 21. In addition, applied polynomial regression analysis, showed a positive linear regression between the values of H/G % against the bacterial growth diameter as shown in Fig. (3). Moreover, another positive linear regression was also obtained by plotting H/G % values against the diameter of hydrolysis zone as clearly shown in the same Figure.

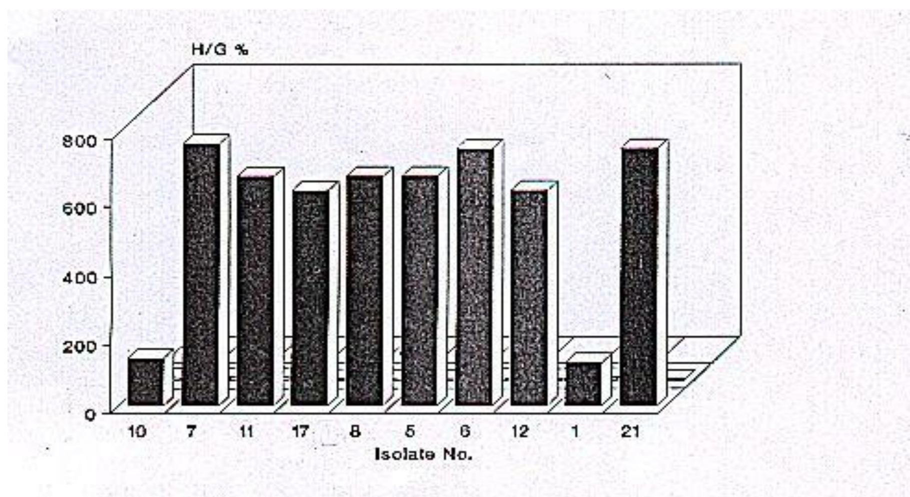

Fig. (2): Screening of bacterial isolates based on H/G %

III- Chemical description of the curing solution:

The chemical description of the curing solution was followed during the whole process. The optical density was measured at 475 nm which adjusted to zero at the first day of the process as control. Gill and Newton (1977) followed the microbial growth in meat juice by measuring the change in optical density at 550 nm. In the experiment here, the maximum reading of the optical density was at the 9th day being 1.08 followed by 0.916 at the 10th day as can be seen in Table (3). Interestingly, these results are quite well to those obtained for examined protease and lipase as well (El-Fadaly *et al*., 1998). The values of pH are ranged between 6.83 to 7.27 as shown in the same Table.

Regarding the total nitrogen (%), the maximum value was recorded at the 9th day being 0.27% but the 8th day come second by 0.26% as shown in Table (3). Again, the values of total nitrogen are in agreement with those values of pH as well as the optical density. The value of total protein was 1.688 after the 10th day while 1.625 was found after the 8th day. The change fold of these three parameters are calculated and their values are also listed in Table (3). The change fold of pH values seems to be constant since it ranged between 1.0 and 1.1, started from the second day. Identical change fold was observed with values of total protein which gradually increased up to the 9th day. This may be due to dehydration.

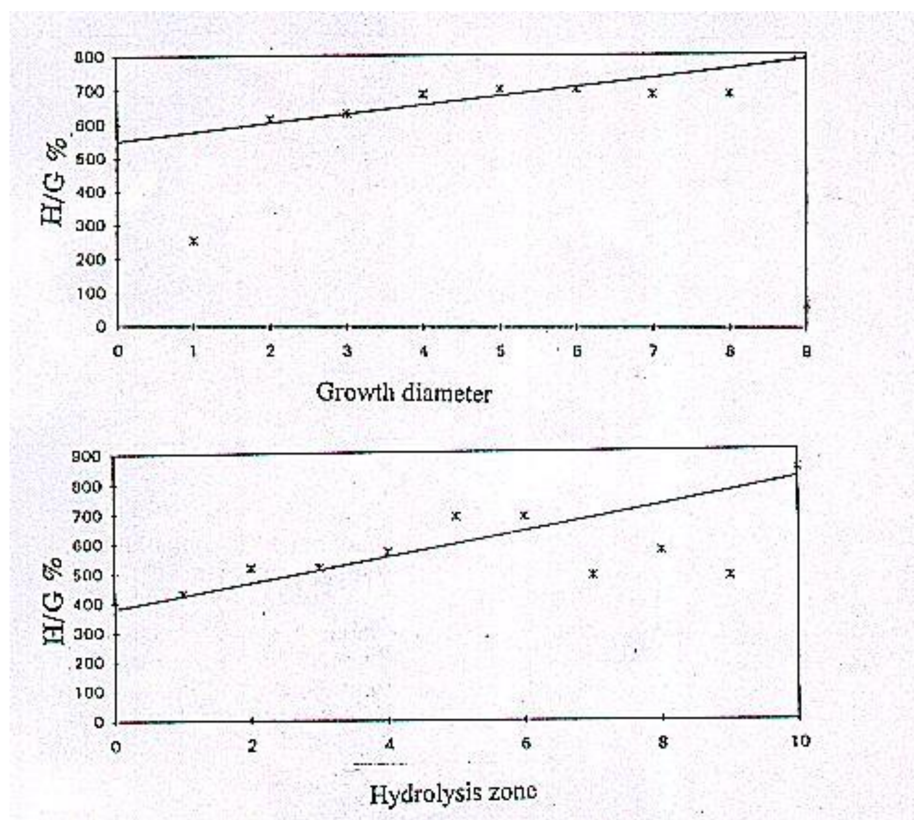

Fig. (3): Linear regression of H/G% against growth diameter of the bacterial isolates and hydrolysis zone.

Table (3): Chemical description of the curing solution during the process of treatment.

Treatment				Total protein	
period (days)	OD*	pH	change fold	%	change fold
1 (Control)	0.00	6.83	-	0.438	-
2	0.176	6.95	1.0	0.813	1.9
3	0.260	7.27	1.1	0.813	1.9
4	0.275	7.09	1.0	1.063	2.4
5	0.440	7.03	1.0	1.188	2.7
6	0.533	7.04	1.0	1.313	3.0
7	0.578	6.91	1.0	1.438	3.3
8	0.590	7.12	1.0	1.625	3.7
9	1.080	7.21	1.1	1.688	3.9
10	0.916	7.01	1.0	1.438	3.3

*** Optical density at 475 nm.**

IV- Values of sugars measured in the curing solution:

During the whole process, the measurement of different forms of sugars were quantitatively traced. The values of reducing sugars gradually increased from start of the process to the end being from 0.051% to 0.161%. High value was observed after the 10th day being 0.161. This followed by 0.141 at the 9th day as can be seen in Table (4). This increase of reducing sugars can be explained by the fact that these sugars considered as products of the microbial action against the high molecular weight molecules present in the curing solution.

Table (4): Values (%) of sugars in the curing solution.

Treatment	Reducing sugars		Non reducing sugars		Total sugars	
period (day)	%	change fold	%	change fold	%	change fold
1 (Control)	0.051	-	4.40	-	5.00	-
2	0.058	1.1	4.20	0.9	4.46	0.9
3	0.078	1.5	3.39	0.8	3.47	0.7
4	0.084	1.6	3.74	0.9	3.83	0.8
5	0.109	2.1	3.39	0.8	3.50	0.7
6	0.115	2.3	2.56	0.6	2.68	0.5
7	0.125	2.5	4.23	1.0	4.36	0.9
8	0.136	2.7	4.42	1.0	4.56	0.9
9	0.141	2.8	3.55	0.8	3.70	0.7
10	0.161	3.2	4.06	0.9	4.23	0.9

In contrast, the values of non reducing sugars are quite differentiated along the whole period of meat treatment. The dynamic of these forms may be because of the microbial action on these types of sugars as energy and carbon sources. The maximum value was found at the 8th day followed by that after the 7th day compared to the value of control which recorded at the first day as demonstrated in the same Table. The values of total sugars were also considered. These values showed to be differentiated along the period of meat curing. It could be noticed that the value of total sugars decreased up to the 6th day then increased up to the end of the treatment. Additionally, the change fold of these different forms was also calculated. Results exhibited a well fit between both non reducing sugars and total sugars whilest the change fold of reducing sugars showed to be higher and showed gradual increase, too.

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وصف التغيرات الميكروبية والكيماوية خلال عملية تمليح اللحم

حسين الفضالى ** - محمد قاسم ** - محمد حامد* وفاتن حسن*
قسمى الصناعات الغذائية* والميكروبيولوجى - كلية الزراعة - جامعة المنصورة - المنصورة - مصر ٠**

تم إجراء فحص ميكروبيولوجى لكل من محلول التمليح وعينات اللحم المعاملة بإستخدام بيئات زرع مختلفة، وذلك بجانب عمل برنامج تصفية للعزلات البكتيرية الناتجة بإستخدام بيئة آجار اللبن وذلك لتحديد كفاءتها فى تحليل كازين اللبن٠ كذلك تم متابعة الوصف الكيماوى علاوة على تقدير السكريات فى محلول التمليح٠ أوضحت النتائج أن كل من اللحم ومحلول التمليح تختلف فيما بينها من الناحية الميكروبيولوجية والكيماوية٠ كذلك وجد أن هناك علاقة خطية موجبة بين قيم H/G % وقطر النمو الميكروبى للعزلات البكتيرية الناتجة وكذلك بين قيم H/G % وقطر الهالة الناتجة من تحليلها لكازين اللبن٠ بينت كل من قيم pH والكثافة الضوئية لمحلول التمليح قيما ثابتة خلال عملية التمليح٠ بينت النتائج أيضا أن قيم البروتين الكلى فى زيادة تدريجية حتى اليوم التاسع فى حين أنخفضت قيم السكريات الكلية والسكريات الغير مختزلة حتى اليوم السادس، بينما كانت قيم السكريات المختزلة فى حالة ثابتة خلال عملية التمليح والتى إستغرقت عشرة ايام٠