The effect of storage on the Microbial Evaluation to some kinds of meats in the local market at kerbala city.

التقييم المايكروبي لبعض أنواع اللحوم في السوق المحلية لمدينة كربلاء المقدسة ، وتأثير الخزن عليها

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Abstract

This paper contains a study of the microbial content of the cow carcass and minced meat in butcher shops in Karbala city from 1-April-15May.The study also deals with the influence of normal storage under cold conditions and bad storage (25c° room temperature) in Iraqi current conditions on the development of the number of microbes under study which included: the aerobic plate count (APC), count of Total coliform (TC.), the number of ***staphylococcus aureus*** , the total number of mold & yeast (M &Y). Checking the presence of ***Salmonella*** bacteria and also checking the Hydrogen number (PH) For the meat samples. It is noted that more than 66% of the cow carcass and the minced meat samples have shown that their negligence of international properties as regards the (APC) and the (TC).While the number of ***staphylococcus aureus*** were unaccepted in 50% and 30% of the cow carcass and minced meat respectively .As regards the molde and yeasts (M&Y), all meat samples were counter to the international properties and that's the evidence of meat contamination in the external environment. The ***Salmonella*** bacteria were present in 66% of the cow carcass and in 33% of the minced meat. There was no significant effect in the increase or reduction of the presence of these bacteria for both types of meat whether storage under 5C° or 25C° .As regards the other microbial numbers , they developed remarkably and signs of perceptible damage for both types as well as storage temperature have been demonstrated and this is owing to the rise of the primary numbers for these tests and also due to the negligence of proper hygienic conditions that slaughterhouses should abide by, the slaughtering outside slaughterhouses, and the absence of hygienic control.

الخلاصة

تضمن هذا البحث دراسة المحتوى المايكروبي للحم ذبائح البقر وكذلك لحم البقر المثروم في محلات القصابين لمدينة كربلاء المقدسة للفترة من ١/ ٤ / ٢٠٠٥ ولغاية ١٥ / ٥ / ٢٠٠٥ وكذلك تم دراسة تأثير الخزن الاعتيادي تحت التبريد والخزن الردئ ٢٥م^{o} (Room temp).في ظروف العراق الحالية على تطور الاعداد المايكروبية قيد الدراسة والتي تضمنت : العدد الكلي (APC) للبكتريا الهوائية ، العدد الكلي لبكتريا القولون (TC) ، عدد بكتريا المكورات العنقودية ***Staph . aureus*** ، العدد الكلي للخمائر والاعفان (M.y.) والفحص عن تواجد بكتريا التيفوئيد (السالمونيلا) وكذلك فحص الاس الهيدروجيني (PH) لعينات اللحم .تبين ان أكثر من ٦٦ % من النماذج لحم الذبائح واللحم المثروم الطازج مخالفة للمواصفات العالمية من حيث اعداد (APC)، (TC) ، اما اعداد بكتريا ال ***Staph . aureus*** فكانت مرفوضة في ٥٠ % ، ٣٠ % من نماذج لحم الذبائح واللحم المثروم على التوالي ، اما بالنسبة لفحص الخمائر والاعفان (M.y.) فكانت جميع نماذج اللحم مخالفة للمواصفات العالمية وهذا دليل تلوث اللحوم من المحيط الخارجي ، اما بكتريا التيفوئيد فكانت متواجدة في ٦٦ % من نماذج الذبائح الطازج و٣٣% من نماذج اللحم المثروم ولم يكن هنال اي تأثير معنوي في زيادة أو نقصان تواجد هذه البكتريا لنوعي اللحم سواء في الخزن على ٥ م^{o} أو ٢٥ م^{o} .أما اعداد الاحياء المجهرية للأنواع الأخرى فقد تطورت بشكل معنوي ملحوظ وظهرت علامات التلف الحسي لنوعي اللحم وعلى درجتي حرارة الخزن وهذا يعود أرتفاع الاعداد الأبتدائية لهذه الفحوصات بسبب عدم تطبيق الشروط الصحية الواجبة في المسالخ وكذلك عملية الذبح خارج هذه المسالخ وغياب الرقابة الصحية عن محلات بيع اللحوم .

Introduction

The Meat in general is currently looked as one of the most important food stuff all over Iraq .This is ascribable to the constant demand on the part of the customer owing to the increase of population and the increase of the income average in Iraq where the red meat constitute 70% of the gross production in Iraq (Hoshyar,1978).

Red meat and its products are composed of complex components of tissues that are formed of important nutritional elements for human beings like proteins 18%, fat 21%, minerals 0.9%, vitamins(thiamin 0.08%, riboflavin 0.16%, nicine 4.3mlg./100grm)., moist 61%, hydrogen Exponent.(5.5-6.8), according to (Weiser *et al*,1971).

The protein in meat contain a high biological value because it contains all essential amino-acids required to build and maintain its activity, and that the taste of red meat is desired since it has a highly dynamic role in activating the biological operations in the body.

Meat is regarded as a suitable medium for the growth and proliferation of microbes on account of its being full of nutritional elements, high moist, and suitable hydrogen\ number (,.It is noted that internal part of fresh meat has no microbes but it might contain little of them in the lymph nodes in the bone marrow.(Fraizer,1978).One can notice from the ratios of meat components that the carcass meat is considered a food stuff that is good for the growth of microbes where this meat does not contain a perceptible quantity of carbohydrates .the malign microbes in meat is of the type of proteolytic analysis where they get both the nitrogen and carbon from the amino-acids. Minced meat is regarded as most suitable for the growth of microbes in meat due to release of biles in the tissues and the increase in the superficial area in it when it is minced and consequently the microbes will be in contact with the original meat components where they cause its damage quickly as a result of the spread of microbes within the meat tissues and that the contamination speed of the microbial proliferation depend on the quality of the meat , the ways it is handled, the cleanliness of the butcher tools in mincing,storage temperature, and the cleanliness of workers.The main sources meat contamination are soil, water, and dust. Animal leather is considered to be the chief medium for transmitting such microbes, notably aerobic and anaerobic bacteria, to the meat via:

1-Knives and other tools used in slaying , skining, and knifing animals.

2-the contact of workers' dirty hands and clothes with meat.

3-The contamination of meat through the contact of meat with the animals offal during slaughtering(Muslih,1990)When the bacteria grow on the meat surface, a sticky matter with a dark or bright color appears notably on beef in the neck where the knife cuts the body and the animal's front legs because of the floating bacteria there.The bad smells emanating from the rotten meat are ascribable to the emission of gases including H_2S and acids due to the metabolic results during the growth of bacteria.When a perceptible rot appears , this will be a sign of the increase of bacteria number has mounted up to($50\text{-}100\times10^6/cm^2$) on the meat surface or per gram.Big chops of meat i.e. a quarter or half of the carcass is less liable to rot than small pieces and these in turn will have a longer storage period than minced meat because of the area length exposed to the bacterial growth.The surface of bigger chops is comparatively dry and covered with fats and the presence of bacteria is superficial while in smaller bits of meat become more liable to contamination owing to the exposure of a wider area to microbial pollution notably in minced meat where the bacteria mingle with meat during the chopping process.

The most significant factors that influence the meat rot bifurcate into**:**

A-Factors related to the animal before and after slaughtering.

1-The content of animal Rumen: When the microbial number increase; the meat contamination will increased there for the animal should fast for 24hrs. befor slaughtering.
2-The physiological condition of the animal: when an animal is slaughtered while it is fatigued, the lactic acid becomes more concentrated on account of the transformation of glycogen into a lactic acid and consequently the (PH.) decreases which in turn limits the growth of microbes during storage.
3-Bleeding during slaughtering: the more the animal bleeds when slain, the longer the period of his storage because of the meagre quantity of microbes for blood and lymph are the media for carrying and growing microbes in tissues.
B-Factors related to meat storage:
1-The type and degree of microbial contamination in meat: the increases of primary number of microbes the less the period of storage. The type of polluting microbes is significant because if the microbes are psychrophiles, they will play havoc on meat more.
2-Physical and chemical properties of meat: the bigger the exposed area to the outside world, the quicker it rots .Minced meat is more exposed to the pollution of the outside world. The presence of fat layers covering meat protects the meat against microbial rot but they dissolve as an enzyme and oxidize speedily.
Factors related to meat properties:

1-Moist: moist is a significant factor because superficial dryness diminishes the number of microbes. The degree of water activity identifies the nature of microbial growth in meat. Generally, microbes causing rot need high humidity.

2-PH.: it ranges in beef from 5.1-6.8 while in mutton ranges from 4-6.7 and this depends on the quantity of glycogen in the animal body and its physiological condition before slaying. rot-causing bacteria favor the neutral PH.

3-Oxygen: the presence of oxygen promotes the growth of aerobic bacteria and its absence in canned food makes the rot because of the anaerobic bacteria.

4-Temperature degree: when meat is stored, psychrophiles-able to grow and proliferate-are the reason for meat rot. The lower the storage temperature degree, the longer the period of stasis in bacteria and consequently prolongs the period of storage without causing rot of meat.

Materials and Methods

A-Meat samples:
1-Cow carcasses meat
2-Minced cow meat
B-Culture media
1-Agar-Agar(Rashmi Diagnostic)
2-Brilliant Green Agar(Himedia)
3-Lactose Broth(Himedia)
4-Nutrient agar(Acunedia manufacturers)
5-mannitol salt agar(Himedia)
6-MacConky Agar(Rasshmi Diagnostics)
7-Triple Sugar Iron Agar(Microbiological)

8-Potato Dextrose Agar(Oxoid) :Add(0.05)gm/liter of Chloromophenicol as antibacterial agent (Kwon-chung,K.J.&Bennett,J.E.1992)

Note:All of the culture media were sterilized by Autoclave,at 121c for 15 min.

D.Procedures:

1-Collecting and preparing samples:

(6) samples of cow carcass and minced meat have been collected for the period 1/April-15/May-2005 from local markets in karbala in sterilized plastic sacs where they were directly transported to the lab and kept in a refrigerator. The samples were tested immediately because all requirements were prepared in advance to avoid the growth and proliferation of microbes in these samples.

The used samples are taken to be checked and sliced into very small pieces using a sterilized knife and a forceps .Pieces are taken from different parts of the carcass .As for the minced meat, a piece is taken and put on a sterilized plate then it is spread in the form of a circle.Afterwards , parts of the circumference and middle are taken then they are mixed well till they become homogenous meat mixture .Each sample will wigh (11gm) where (99ml) of distilled water added and the sample will be mixed by a stomacher for (3) minutes with the maximum speed ,then the mixture is left for (15) minutes, after that the emulsion is stirred calmly for the purpose of amalgamation .Ultimately the required dilutions are made for the microbiological tests(Al-Dulaimy,1988).

2-Storage of Samples:

Microbiological tests have been made when the samples were brought (at once) and part of the sample were stored in the refrigerator under (5+1)C° because it is the degree under which butchers store their meat in the local markets.Other samples were stored under room temperature(25-30)° because this a bad degree for storing meat when the power is off and this is a common condition in the Iraqi markets.Microbiological tests have been made after 48hrs. of storage.

3-Microbial Tests:

A-Aerobic Plate Count(A.P.C.)

The above-mentioned method was used by (Nackerson & Sinskey, 1977) by using pour plate technique and the nutrient agar,and count the colone/gm from sample after incubation period 24 hrs. in35-37c.

B-Total Coliform(T.C.)

The same previous method was used on the (MacConky agar) and the colonies were counted after a (24)hrs. period under (37)C° per gm. Of the samples big colonies with pink, red color and mineral glitter.

C-S.aureus

The afore-mentioned method was used by(BBL-1973) using streaking method on the stiffened Mannital salt Agar in plates then incubated under (37)C° for (48) hrs.After that the number of distinguished golden yellow-aura colonies in the plate is counted

D-Salmonella spp-

1-Presumptive test for ***Salmonella***

This test was conducted by (Nackerson & Sinskey,1977) via the amalgamation of (25gm) of sample to be tested with(225) ml of the enrichment media i.e.(lactose broth) in a (stomacher) with a maximum velocity for (3) minutes then the emulsion is put in a sterilized flask.It is then incubated under(37)C° for (24)hrs. and planted according to the streaking method on the stiffened Brilliant green agar in a plate.it will afterwards be incubated for (48)hrs. under (37)C° where the probability ***salmonella*** colonies will appear on the transparent pink colonies surrounded with red-aura.

2-Confirm test for ***Salmonella***

This type of test is used to confirm the presence of ***salmonella*** the Triple Sugar Iron agar in the form of (slant) and inculated from pure colonies by streaking method and (stab).the appearance of the black color in the bottom of the tube(proof of the production of H_2S) and the transformation of the medium at the top of the tube from red to yellow(proof of the production of an acid)(BBL,1973)

E-Molds & Yeasts:

The number of molds and yeasts was estimated by using(pour plate) method where the (potato dextrose agar) was used adding 0.05gm/litre of chloromophenicol after sterilization as a anti-bacterial growth as it is shown before where plates were incubated under (22 + 2)C° for (5) days.Finally,the number of colonies was counted per each gm of the sample.

F-The measurement of the Hydrogen Exponent (PH):

The method explained in (APHA,1958)was used where the PH for the samples is measured with a PH.meter by mixing(11)gms of the sample with(99)ml of distilled and sterilized water and mixed well in the stomacher for(3) minutes then the PH for the sample is measured.

CRD was used to find the effect of storage on the studies proprieties .Duncan was applied compare significant differences between means

(AL-Rawy&Abdul-Azez,1980)

Results and Discussion

The Microbial Quality for some Types of Meats:

Microbiological tests have been conducted including(APC), (TC),(M.Y),S.aureus, and the probable test for the presence of typhoid bacteria(salmonella) for the cow carcass meat and also cow minced meat.One can observe in table(1) and (2) that the average of (APC) for the cow carcass meat is 5.7×10^6/gm.These counts are incompatible with the international and Iraqi properties. Thus the tested meat is contaminated and the growth of bacteria, the time necessary for the damage of meat depend on the degree of primary contamination with these bacteria, and the period meat remains in the markets and their storage (Prescolt & Dunn,1982).The pursuit of hygienic methods in dealing with meat before having it chopped i.e. the washing of the carcass and sterilizing tools are factors that lead to the lessening of (APC).While in the minced meat the average of the (APC) is 4.35×10^7/gm.This average is considered incompatible with the international and Iraqi properties and the mincing of meat, release of bile, the exposure for blood remains and polluted parts, the increase of the superficial area exposed to bacteria, and the

contact of contaminated parts with the unpolluted parts are all factors conducive to the increase of (APC)The Total Count for the coliform bacteria was in cow carcass meat 2.4×10^7/gm while in minced meat was 10.7×10^7/gm.These results are incompatible with the international and Iraqi properties. The presence of coliform bacteria is a proof of contamination with human and animal remnants which are manifest either in the form of excreta or else like air,soil,and insects.It is also a proof of unhygienic slaughterhouses, the tools,machines,and the workers' clothes.The rise of (TC.) constitutes a hygienic peril because their presence is another proof of the presence of phathogen bacteria .

(Rogers & Mcdeskey, 1957),refered to the disconnection of between the increase and decrease of Aerobic plate count total coliform which reflects the obvious difference within themicrobiological tests as in tables(1)and(2) for the counts of (APC) and (TC.) It is found that the average of ***S.aureus*** numbers is 1.11×10^5 cell/gm meat and 5.33×10^4 cell/grm. Meat for the carcass meat and minced meat respectively as is explained in table (1) and (2).These results indicate the meat pollution and its inconsistency with the international properties anmd its various sources of pollution like air, meat transportation, the suitable conditions like moist,the availability of nutritional elements,the physical contact for the workers, and the abus
e of apparatus and equipment.

(Al-Ghizzi,1985) has discovered that the average of S. aureus in minced meat is 0.9×10^5 cell/gm meat .This what (Al-Delimy & Stiles,1975) found where the ratio of samples in which the number surpassed 1×10^4 cell/gm meat is 51%.The researcher (Hoshyare *et. al,*1982) found that 85% of his samples surpass that number.As regards the Salmonella and according to tables (1) and (2), it is found that 67% and 33% of the carcass samples contains this type of bacteria which indicates the pollution of these samples and their inconsistency to international properties that do not tolerate the presence of this bacteria at all.This is an evidence of the exposure of meat to infected people or carriers for this bacteria whioch should not be present in all types of food prepared for human consumption.These results conform to what have some researchers found out(Turnbull& Rose,1982), (Al-Ghizzi,1985)The presence of Salmonella led to cases of poisoning for 12 persons in Riadh city due to eating canned beef(Nabbut *et. al,*1982).The sources of infection are the workers,butchers' shops, breeding fields,slaughter houses, and the tools used for butchering and mincing.As regards the molds and yeasts,the averages are found to be 5.68×10^6 cell/gm meat and 7.95×10^4 cell/gm meat for the carcass meat and the minced meat respectively.These tables are regarded high compared to other similar studies where the averages were 5.8×10^3 cell/gm minced meat(Ali *et. al,*1982).The averages were 5.6×10^2 cell/gm minced meat as is found by(Al-Delimy and Stiles,1975)The high numbers of the molds and yeasts are regarded an evidence for the exposure of the meat to the contaminated outer environment with the spores of molds and yeasts which may not constitute a sanitary risk compared to other types of microorganisms but they are considered an evidence for the pollution of the outer environment.

Tabel : 1 Means of microbial number / gm fresh cow carcass meat , And the number after 48 hrs.storge in $5c^o$ and $25c^o$

Treatment	APC	TC	*Staph.aureus*	Mold &Yeast	*Salmonella*	pH
Fresh carcass meat Static symbol	5.7×10^6 B	2.4×10^7 B	1.1×10^5 B	5.68×10^6 A	67% A	6.7 A
Storage 5 c°for 48hrs Static Symbol	6×10^7 AB	6.7×10^7 A	6.2×10^5 AB	18.8×10^6 AB	67% A	6.5 B
Storage 25c°for 48hrs Static Symbol	3.8×10^8 A	1.8×10^8 A	12.5×10^6 A	13.6×10^7 A	50% A	6.25 C
Significanty	**	**	**	*	NS	**

*: p (0.05)
**: p (0.0 1)
NS: Non significant

Table 2 : Means of microbial number / gm of cow minced meat , and the number after 48hrs. storage in $5c^O$ and $25c^o$.

Treatment	APC	TC	*Staph.aureus*	Mold &Yeast	Salmonella	pH
Fresh minced meat Static symbol	4.35×10^7 A	10.7×10^6 A	5.33×10^4 B	7.95×10^4 B	33% A	6.5 A
Storage 5 c°for 48hrs Static Symbol	8×10^7 A	10.6×10^7 A	6.67×10^6 A	4.88×10^5 A	33% A	6.5 AB
Storage 25c°for 48hrs Static Symbol	4×10^8 A	2.65×10^7 A	3×10^6 A	2.93×10^6 A	16% A	6.3 B
Significanty	NS	NS	**	**	NS	**

*: p (0.05)
**: p (0.0 1)
NS: Non- significant

Microbial Changes for Meat during Storage:

As regards the cow carcass meat , it is noticed that in table (1) and (2) the (APC), ***Staphyccococus aureus***, and molds and yeasts, the increase in their numbers was not of an significant value after storing for (48)hrs. under 5C° except for the number of (TC) bacteria where the average reached 6.7×10^7/gm.But there was a remarkable increase in the number of bacteria included in the test after storage for (2) days under (25)C° and the appearance of a perceptible damage when anaerobic bacteria (APC)reached 3.8×10^8/gm,the TC 1.8×10^8/gm, the ***S.aureus*** 1.25×10^7/gm, and the molds and yeasts 1.36×10^8/gm.These results agree with what the researcher (Jay,1962) arrived at where he pointed out that the damage occurs in stored meat when the (APC) reaches 10^8-$10^{9/gm}$ .Results indicate the decrease of salmonella rate up to 50% in samples of stored meat under room temperature because is type of bacteria is considered a Fastidious Heterotrophs where the moist in meat decreases and the acidity in meat increases where the PH in 6.7 of fresh carcass meat after storage under 5C° to 6.2 in stored meat samples under 25C° and that's to increase the results of metabolism(acids) after the development of many other kinds of microbes and these factors lead to the decrease of ***salmonella*** numbers.As regards the minced meat, there was an increase in the numbers of ***S. aureus*** and Molds and Yeasts where they reached up to 6.67×10^6/gm , 4.88×10^5/gm respectively when stored for (2) days under 5C°.This is an evidence for the suitability of this degree for the growth of this type of microbes and also an evidence for the contamination of these samples from the outside invirenment.There was no significant increase in the number of (APC) which is a proof that the great part of the bacteria was formed of mesophiles where its growth becomes restricted when stored under 5C° .
The increase in (APC) and (TC) is not significant when stored under 25 C° for 48 hrs. where they reached 4×10^8/gm,2.65×10^7/gm respectively.
It seems so far that the results of samples testing for the carcass meat and minced meat found in local markets of Karbala city are considered as polluted and against the international and Iraqi properties for the consumable meat by human beings and thus the storage process affects the increase of microbes numbers and so we recommend the following:
1-The strengthening of sanitary surveillance over all facilities that are related to public health and also the periodical checking for workers and slaughtering tools and machines therein.
2-The pursuit of sanitary conditions in slaughterhouses by preventing the entrance of animals and insects like dogs and cats to these places.
3- Never quarantine sick animals with sound ones lest they contract a contagion.
4-The starvation of animals before they are slain in order to lessen the microbial content in the intestine.
5-The adherence to good and quick methods of slaughtering so that the animal bleeds the biggest quantity of blood because having too much blood in the animal tissues will spoil it.
6-The preservation of meat under suitable temperature degree and transporting it in the same manner to the shops.
7-The assignment of severe sanitary control over animal breeding pastures.

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