بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Veterinary Medicine and Animal Production

Department of meat Science and Technology

Hygienic Conformity of beef sausage collected from different sources in Khartoum state with SSMO Specification

صحية السجك البقري بولاية الخرطوم ومدي مطابقته لمعايير المواصفات السودانية

Thesis submitted in partial fulfillment for requirement of B.Sc. (Honor) degree in Veterinary Medicine and Animal Production(meat Science and Technology department)

By

Asma Abed Albage Yousif Alidros

Rofyda Ahmed Mohammed Abakar

Omima Faroug Alawad Mohammed

Supervisor:

Professor Dr. Daouood Alzuber Ahmed

July 2014

الكلمات المفتاحية

١/ التطابق Conformity

٢/ صحية Hygienic

٣/ السجك Sausage

٤/ معايير Specification

بسم الله الرحمن الرحيم

قال تعالي :-

﴿فمن كان يرجو لقاء ربه فليعمل عملاً صالحاً ولا يشرك بعبادة ربه أحد﴾

صدق الله العظيم
[سورة الكهف الاية ١١٠]

إلي نبع الحنانوفيضه الرقراق أمي،،،،

إلي سندي ومصدر قوتي أبي،،،،

إلي من أضاءوا لي الطريق

وسط عتمتي وضلالتي، أساتذتي

إلي شمعتي التي تحترق دوما

لكي تضيئي لي ولاتنطفئ مشرفي

إلي من بهم أسعد وأضحك وأبهج

أسرتي،أحبائي،أصدقائي

إلي كل من شارك وقدم لي معروفا

لكم جميعا أهدى مجهود سنينا من عمري

أهدي لكم قليلا من علما إنتهلته

لعل العلم أفضل ما يهدي به المرء

الشكر والعرفان

لا بد لنا ونحن نخطو خطواتنا الأخيرة بالحياة الجامعية من وقفه نعود إلي أعوام قضيناها في رحاب الجامعة مع أستاذتنا الكرام الذين قدموا لنا الكثير باذلين بذلك جهدا كبيرا؛لبناء جيل الغد لنبعث من جديد....

وقبل أن نمشي نقدم أسمى آيات الشكر و الامتنان والتقدير والمحبة ألي الذين حملوا أقدس رسالة إلي الحياة...

إلي الذين مهدوا لنا طريق العلم والمعرفة...

ألي جميع أساتذتنا الأفاضل...

"كن عالما..فإن لم تستطيع فكن متعلما،فإن لم تستطيع فأحب العلماء،فإن لم تستطيع فلا تبغضهم".

نتقدم بوافر الشكر والتقدير للأستاذة /وصال عباس

حفظها الله ورعاها

التي قامت بإرشادنا وتوجيهنا في جميع مداخل هذا البحث ،،،،،،

ونخص بالشكر والتقدير أستاذ/محمد سر الختم الذي مدنا بالمعلومات اللازمة لإتمام هذا البحث المتواضع

كما نتقدم بالشكر والتقدير لجميع الصديقات والأصدقاء الذين قاموا بمساعدتنا في الحصول على المعلومات الهامة .

وكذلك نتقدم بالشكر لجميع أساتذة قسم علوم وتكنولوجيا اللحوم علي مقدمتهم بروفيسر داؤد الزبير،،،،

وشكر خاص جدا للدكتور حيدر الأمين الذي ساعدنا في إتمام هذا البحث المتواضع ،،،

List of contents

List of tables :-

الـمستخلص

أجريت هذه الدراسةبمعمل الأحياء الدقيقة – كليه الطب البيطري بجامعة السودان للعلوم والتكنولوجيا في الفترة من ٢١/٥ــــ٢١/٤ والهدف من هذه الدراسة التعرف علي مدي تلوث عينات السجك الطازج بالسامونيلا والأشريشية القولونية المأخوذة من ثلاث مصانع مختلفة بولاية الخرطوم (أ،ب،ج) وكذلك لتقدير العدد الكلي للبكتريا في العينات الذي كان في :-

$6,06\times10_{5}$ و$5,99\times10_{5}$ و$6,08\times10_{5}$ علي التوالي.

وان العينات غير مطابقة للمواصفات والمقاييس السودانية للعام ٢٠٠٨.

Abstract

This study was conducted in microbiology laboratory ,college of veterinary medicine and animal production ,Sudan university of science and technology ,during 21 April -21may 2014 .The aim of this study is to investigate if the samples of fresh beef sausage which taken from three different factories in Khartoum state coded A,B,C were contaminated with salmonella and E.colibacteria .The total bacteria count of samples A'B'C was found to be $5{,}99x10^{-5}$,$6{,}0x10^{-5}$ and $6{,}08x10^{-5}$ the bacteria count contaminant with E.coli and salmonella were not inconformity with Sudanese standard metrology and organization (SSMO) 2008 specification.

Chapter one

1.1. Introduction:

Meat is considered as an important source of proteins, essential amino acids, B complex vitamins and minerals. Due to this rich composition, it offers a high favorable environment for the growth of pathogenic bacteria. The microbiological contamination of carcasses occurs mainly during processing and manipulation, such as skinning, evisceration, processing, storage and distribution at slaughterhouses and retail establishments. Fecal matter is a major source of contamination and can reach carcasses through direct deposition, as well as by indirect contact through contaminated, equipment's, workers, installations and air (Borch 2002).Good ManufactoryProcess (GMPs)are programming that comprise the basic, universal steps and procedures that control operating conditions within establishments and ensure favorable conditions for the production of safe foodHACCP systems relate to hazards within a specific process. GMPs are the control factors that relate to the entire operation and are not process-specific. GMPs include such programs as pest control, recall procedures, construction/maintenance and sanitation. In order to ensure that GMPs are carried out, there are step-by-step descriptions that instruct individuals as to how, when and what tasks are to be performed for a required GMP. The FSIS food safety inspection sanitation Pathogen Reduction/HACCP rule published in July, 1996 combines the concepts of Hazard Analysis and Critical Control Point systems with the FSIS requirement for written Sanitation Standard Operating Procedures (SSOPs). In order to avoid confusion, it is important to have a clear understanding of how the HACCP concepts relate to SSOPs as well as the relationship between the SSOPs, Good Manufacturing Practices (GMPs), and HACCP plans that many companies already have in place (American meat institute foundation 1997). Over the last 15-20 years, there has been a general increase in the number of cases of food

borne illness, although there has been a recent decrease in the number of food poisoning cases due to Salmonella. Refer to Figure 1, which illustrates the rise in laboratory reports of food poisoning cases, Salmonella and E.coli.

1.2. Research problem:-

1. Increases in food poisoningdue to consumption of contaminated meat products.

1.3. Researchobjectives:-

2. Toinvestigate whether thesausage under study with inconformity with the SSMO specification.
3. To evaluate the level of contamination of the beef sausage samples.
4. To study the contamination organism (bacteria) in the sausage under the study.

Chapter two

2.1 Literature Review

Food borne microbial hazard may be responsible of many cases of food poisoning each year and that is an important food safety challenge .To lower the incidence of food borne disease ,many experts and stakeholders urge the development of which decision makes prioritize hazards and inter venations using the best available data on the distribution requires an understanding of the many risk factors from point of producing.

Thesausage:-

The sausageis the meat product that produced fromfresh, child,salted or dried meat that packed in clean hygieniccasing.

Types of sausage:-

1. Fresh sausage**.**

Made from ground meat which are seasoned and stuffedinto casings.

2. Cooked sausage.

Made from ground meats which areground, seasoned. Often cured stuffed into cooked casing.

3. Cooked smoked sausage.

4. Uncooked smoked sausage.

5. Dryand semi-dry sausages.

2.2: Meat decontamination:-

The presence of pathogenic bacteria on the surface of carcasses ,even though of low prevalence , emphasizes the need for proper refrigeration , handing ,and cooking of meat product before consumption .

Study on ground beef for E.Coli O 157:H7from (1994 to 2007) showed an average percentage of less than 0.5% positive samples from federal establishments and retail stores and decreasing trend of E.coli O157:H7 prevalence below 0.20% after 2003,in samples taken for the U.S ground beef testing program (USDA.FSIS2008).

2.2.1 Potential concerns and risks associated with decontamination:-

An important concern of organic acid decontamination is the potential of selection of strains that maybeable to adapt and develop acid resistance.

Subsequently, such strains may colonize equipment surfaces, recontamination carcasses and resist subsequent decontamination treatments (samelis and sofos 2003, 2005).

2.3. Bacteriadecontamination:-

1. Shigella.
2. Vibrio cholerae.
3. Vibrio parahemolyticus.
4. Vibrio vulnificus.
5. Bacillus cereus.
6. Campylobacterjejuni.
7. Yersinia enterocolitica.
8. Salmonella

2.3.1. Salmonella:-

Is the classic example of food borne infectionSalmonella enteritidiswas isolated in 1884, and still is important food borne organism.

In 2002, there were 357 out breaks, with 32,610 cases and 13 deaths due to salmonella reported in the United States.

Salmonella has been found in water, ice, milk, dairy products, shellfish, poultry and poultry meat products, egg products, animal feed and pets.(Fidel Toldra Editor,2010) , He also stated that, human beings can be healthy carriers of this organism.It has been estimated that 4% of the general public carries of this organism, with more females than males being healthy carriers.

Detection of salmonella by the classical method includes pre-enrichment of culture from food samples enrichment or selective enrichment of the liquid culture plating of liquid and selective agar to isolatecultures biochemicaltests of suspect colonies, and confirmation of isolates with typical biochemical profiles by serological tests.

Salmonella is heat sensitive .Proper cooking will destroy the organism .Also proper chilling; refrigeration and good sanitation will minimize the problem. Salmonella remains one of the most important food pathogens in our food supply.

2-3-2.Escherichia coli:-

E.coli is a gram-negative, facultative anaerobic, non-spore forming rod that occurs widely in nature as well as in the intestines of human and animals. It is glucose, and lactose, positive in dole and methyl red positive butvogues proskauer and citrate negative.(Fidel Toldra Editor,2010)

Chapter three

3.1. Materialand methods:-

This study was conducted in microbiology laboratory, college of veterinary medicine SudanUniversityof science and technology, during 21 Aprilto 21 may2014.

Code letters A, B, C samples were taken three times over three weeks, each sample weight 0.5kg.

Preparation of the samples:-

Take 1g from the sample , diluted in 10ml of normal saline

Dilution steps:-

- Take 1ml from prepared sample to 9ml of normal saline in tube (1) , mixed well and tale 1ml from tube (1) to 9ml of normal saline in tube (2) and repeat that step in tube (3,4,5)
- Take 1ml from tube (4) culture it in 2 agar media & incubate in 37c for 18-24 hour
- Count the colony in 2 agar and calculate the mean from them .

Viable count:-

- Plates prepare serial dilution from the sample using finger collation 0.1% peptone water as diluent.

Preparation of dilution:-

- Pipette gal amounts of diluents solution into sterile test tubes with sterile test tubes.

- Mix sample by skating dropsterile 4ml Pipette
- In the sample (only halfalarch) andtake.
- 5ml uses deliver in to the first diluents tube about half and Irish use is the level of the liquid.
- With a fresh pipette dip half an inch into the liquid 1ml and transfer to the next tube.
- Discard the pipette.
- Continue to the required number of dilution and remember to discard the pipette after delivering its contents.(Monica Cheesbrough)

Dilution will be as follows:-

(Tube NO.2)1/100(NO.3)1/100(NO.4)1/10000 (NO.5)1/100000

Plate count:-

- Melt nutrient agar medium tubed in 9 ml amounts, cool to 45C in awater bath.
- Set out petri dishes (two or move for any dilution) and Label with the dilution number.
- Add the contents of one agar tube to each dish and mix as follows:-

Have the dish gently six times in oloch wise circle and repeat counter chook wise .move the dish back and forth six times repeat with to –and from movement .

- Allow the medium to set-incubate at 37c for 24-48 hours.
- To count select plates showing between 30 and 300 colonies.

Calculate the colony count by multiplying the average number of colonies counted per countable plate by the reciprocal of the dilution and report as (colony count per milliliter)..(Monica Cheesbrough)

3.2. Drop count method :- (miles &misera)

In this method small drops of the material are paced on agar plates, colonies are counted in the inoculated areas after in caution.

- Prepare 50 dropper pipettes (the pipettes deliver 0.0 2ml 50 drops per ml).
- Dry plates of suitable medium verywell before use.
- Drop at least five drop of each dilution of sample form ahight of hot move then 2 c each plate (to void splashing).
- Replace the lid –do not more –leave the drupe to dry incubate at 37 c for 24 hours.
- Select plates her wing discrete colonies in drops areas.

Preferably on with gives less that 40 colonies per count the colonies in cash drop .divide the total count by the number of drops counted multiply by 50 to convert to 1.ml .and by the dilution used if the drops un – countable (more than 40 colonies) than the colony count per ml .is more than 1000(COWAN ANDSIEELS 2003)

3.3. Surface count method:-

Used for rough estimate of bacterial number in same samples E.G .in urine examination.

- Place 0.1 ml of sample in the centre of well dried plate of suitable medium.
- Spread it with a loop or spreader all over the surface.

Incubate and count colonies. (COWAN ANDSIEELS 2003)

3.4 BIOCHEMICAL TEST :-

Oxidase test:-

Requirement:-

1. Oxidase reagent freshly prepared ……. Reagent no 3.17.
2. An overnight bacterial culture (strain A and B).
3. Glass Petri dish.
4. Wooden stick
5. Filter paper.
6. Benzene flame.
7. Loop.

Method:-

1. Place a piece of filter paper in a clean petri dish.
2. Add to the filter paper of oxidase reagent.
3. By aid of sterile wooden stick transferacolony from strain A and smear it on the filter paper.
4. Repeat step 3 with strain 13.
5. Read your result with in few seconds.
6. Comment on your result and answer the questions.

Citrate utilization test:-

Requirement:-

1. Koser citrate medium in bijou bottle …….. mediumno.
2. An overnight bacterial culture (train A and B).
3. Benzene flame.
4. Loop.

Method:-

1. By aid of sterile loop inoculate 3ml of sterile koser citrate medium with strain A.
2. Repeat step 1 with strain B.
3. Incubate the bottles at 37°c for up to 4 days, examine daily for growth.
4. Record your result, comment and answer the questions.

Ureasetest:-

Requirement:-

1. Urea medium in bijou bottles …….. mediumno.
2. An overnight bacterial culture (strain A and B).
3. Benzene flame.
4. Loop.

Method:-

1. Aseptically inoculate slope of urea medium with strain a using sterile loop.
2. Repeat step 1 with strain B.
3. Incubate at 37°c for 18-24 hours.
4. Examine, record your result, comment and answer the questions.

Culturing on kligler IronAgar:-

Requirement:-

1. Sterile slope of (k I A) in test tubes …… medium.
2. Straightinoculating wire.
3. An overnight bacterial culture (strain A and B).
4. Benzene flame.

Method:-

1. Stop the presterilized wire into(k I A) medium through slope to the end of the tube by one same
2. Nway, before removing streak the surface of the slope by wire.
3. Repeat step 1 with strain B.
4. Incubate the tubes at 37°c for 18-24 hours.
5. Examine, record your resultand comment.

Gram's stain:-

Requirement:-

1. Crystalviolet …… stains NO…….
2. Lugersiodine …… stains NO…….
3. Neutral red …… stain NO…….
4. Benzene flame.
5. Microscope.
6. Ethano195% …… reagent no.
7. Slides.
8. Loop.
9. An overnight bacterial culture (strain A and B

Method:-

1. Prepare athin smear on clean side and leave it to air dry.
2. Fix the smear by gentile heat.
3. Covers the fixed smear with crystal violet leave it to act for 30-60 seconds.
4. Wash of the crystal violet with clean water.
5. Add lugol's 10 dine and leave it to act for 30-60seconeds.
6. Wash of the iodinewith clean water.
7. Decolorize rapidly with ethanol for few seconds.
8. Wash with clean water.
9. Add neutral red and leave it to act for 2 minutes.
10. Wash of the neutral red with clean water .dry the smear examines it under the microscope.

11. Using oil: Immersionobjective.
12. Record your result, comment answer the questions.
    (COWAN ANDSIEELS2003).

Statistical analysis:-

Samples were randomly selected to participate in this study, the result obtained were analyzed using t. test person correlation test and ANOVA test.

Chapter four

4.1.Results and discussion:-

Viable bacterial count:-

Table (4.1) showed no differences between the three samples. All samples were contaminated with salmonella and E.coli :-

Sample	(f.cu)10^{-4}	(f.cu)10^{-5}	**Escherichia Coli**	Salmonella
A	12	17	+ve	+ve
B1	19	10	+ve	+ve
C1	*16*	*16*	*+ve*	***+ve***
A2	18	11	+ve	+ve
B2	21	13	+ve	+ve
C2	17	12	+ve	+ve
A3	23	12	+ve	+ve
B3	22	11	+ve	+ve
C3	25	14	+ve	+ve

Table (4.2): showed that the microbial analysis for fresh sausage samples:-

Sample	A	B	C
Means± DS	$5.99\pm0.12* 10^{-5}$	$6.06\pm0.04*10^{-5}$	$6.08\pm0.08*10^{-5}$
Sig($p<0.05$)	*	**	**

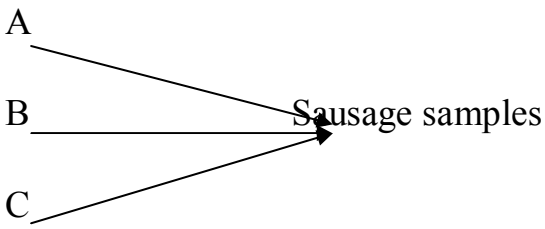

Table (4.3) :shows growth of bacteria in (XLD,MACc) and gram stain :-

N.O	XLD	MACc	Gram stain	Bacteria
1	Black colony	Pale yellow	-ve	salmonella
2	Non lactose ferment	Pink colony lactose ferment	-ve	Escherichia Coli

Primary test:

Table (4.4):- shows the result of primary biochemical test:-

Oxidase	Catalase	O&f	Sugar	Motility	Bacteria
-ve	+ve	f	+ve	+ve	Salmonella
-ve	+ve	f	+ve	+ve	E.Coli

Secondary test:

Table (4.5):- shows the result of secondary biochemical test:-

Indol	Citrate	Urease	Bacteria
-ve	+ve	-ve	Salmonella
+ve	-ve	-ve	E.Coli

KIA:-

Table (4.6):- shows the result of KIA (secondary test):-

Slope	Butte	Gases	H2S	Bacteria
Yellow	Red	+ve	+ve	Salmonella
Yellow	Yellow	+ve	-ve	E.Coli

4.2. DISCUSSION:-

This study showed that there was high significance different ($p \leq 0.01$) in the bacterial count in the sausage samples (B+C) as ($6.06 \pm 0.04 * 10^{-5} \backslash 6.08 \pm 0.08 * 10^{-5}$) where as there was significance different ($p \leq 0.05$) for sausage samples (A) as ($\circ.99 \pm 0.12 * 10^{-5}$).

The bacterial counts in all sausage samples were higher than that of (SSMO2008) specification as (0.25×10^{5}).Where the bacterial count in this study was lower than that reported by (hub &roua2012) as (47.2×10^{6}).The samples of this study were contaminant with E. coli and salmonella which were not conformity with the specification of (SSMO2008) as zero salmonella and E.COLI as (13.8cfu/gm).

Chapter five

5.1. Conclusion:-

This study are weal that the products which were collected from three factories A,B,C is not correspond to Sudanese metrology (2008) in total bacterial count and bacterial contamination ,We strongly recommend the continuation of such research in this field to avail more results

5.2. Recommendation:-

1. Following the goodhygienicpractices during sausage processing.
2. Adequate and appropriate storage and distributionfacilities designed to ensure that no contamination.
3. Good packing of the product.
4. The employers must be provided by changing closes rooms, washingrooms and toilets.
5. HACCP system must be appliedin meat processing centers.

Chapter six

References:-

-Batz MB; DOYLE M.P; morrisJ.G, Painter J; singh R AND Tauxe R.V, Attributing illness to food. Emerging Infection diseases 2005 VO 7, 04 -0634.

-COWAN ANDSIEELS, manual for the identification of medical bacteria (2003) .

- Fideltoldr, an EDITOR, WLEY BLAC KWELL, Hand book meat processing, First published 2010, Black well publishing2010.

- I.J.E.hr, T. L. Tronsky, D.R.Rice, D.M. Kinsman, C. Faustman Home sausageMAKING (2014).

-Monica chees brough, medical laboratory manual for tropical countries.

-Red mead(FAO 2009)

- Sudanese standard metrology and,Organization (2008).

- ماجد بشير الاسود،التجارب ألمختبريه في تكنولوجيا اللحوم(١٩٨٤م).

- هبه مجذوب و رؤى هاشم، جامعة السودان للعلوم والتكنولوجيا ،اثر طريقه التصنيع علي الخواص الفزيائيه والحسيه الميكروبيولوجيه علي السجق ألبقري الطازج(مشروع تخرج٢٠١٢م).