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Effect of Antimicrobial Properties of Pepper Fruits on Some Spoilage Organism of Sudanese Wet-Salted Fermented Fish (Fassiekh) Product

H.M. Adam Sulieman[1*] and Afra A.A. Allaahmed[2]

[1]Department of Fisheries and Wildlife, College of Animal Production For Science and Technology, Sudan University of Science and Technology, P.O Box 204, Khartoum North, Sudan*
[2]Department of Fisheries Science, College of Agriculture and Fisheries, Al- Neelain University, Jebal Aulia, Khartoum Sudan

*Corresponding author's email: hassanadamus@yahoo.com

ABSTRACT

This study was conducted to evaluate the effect of antimicrobial characteristics of hot and sweet pepper on some spoilage organisms of Fassiekh (wet-salted fermented fish) products. The crude Fessiekh (*Hydrocynus spp*) was treated with pepper fruits (fruit-1 *Capsicum annuum* -sweet pepper, fruit-2 *Capsicum frutescens* -hot pepper) as natural conserving materials. The chemical composition of crude and treated Fassiekh were quite significantly different ($p<0.001$) in moisture, ($p<0.05$) in ash and pH, and had no significant differences in both protein and fat. The total viable counts in the first four days after pepper fruit addition were decreased and showed high significant differences ($p<0.001$) between the two types of pepper fruits, and the addition of hot pepper was more effective on the total viable counts which decreased the limits of studied product from $43.4\times10^3\pm 1.3\times10^3$ at first day to $4.5\times10^3 \pm 1.0\times10^3$ after 96 hours, The *Staphylococcus aureus* test showed positive results with count ($7.6x10^3$) for crude, and ($21.9x10^3$) sweet pepper-treated Fassiekh, and negative for hot pepper-treated Fassiekh. The *Listeria spp.* test was found to be positive for Fassiekh treated with sweet and hot pepper, and negative for crude Fassiekh samples and *Staphylococcus aureus* and *Listeria monocytogens* test.

KEY WORDS: Antimicrobial, pepper, spoilage, organisms, fassiekh, wet-salted fermented fish.

INTRODUCTION

The fresh water fishery resources in the Sudan are distributed in an area of about 100.000 km^2, of the Red Sea, which represents the marine fisheries has a coast line of more than seven hundred kilometers Abu Gideiri, (1973). These water bodies constitute a rich source for numerous fresh and marine fish species to many people. Since fish form an important source of human food, the production of its flesh under different natural and artificial conditions is of global commercial interest.

Traditionally, fish and fishery products have been considered to be very safe to eat both absolutely and in comparison to other foods. This view is still correct but a number of events have given rise recently to somewhat more concerned attitude among those responsible for the safety of these commodities. In addition, international agencies such as the World Health Organization and Food and Agriculture Organization have started to pay more attention to this issue. The cause of outbreaks is not always possible to establish, and species, products and practices vary widely between countries and regions, and accordingly intrinsic risks will also vary widely (Connell, 1995). For the latter case a number of preservation methods have been adopted including drying, smoking, salting and fermentation. The process of fish salting and fermentation is termed locally as "Fassiekh" making. Fassiekh is wet salted product, soft in texture with a strong pungent smell and a shiny silvery appearance. It can be stored for more than three months FAO, 1992). Geographically, the industry started in the White Nile Region especially at Gebel Aulia Reservoir about 40 km south of Khartoum, which is the major source of Fassiekh. Other dam reservoirs contributing to Fassiekh output include Sennar, Lake Nubia, and Khashm El Girba Reservoir. Beside Jebal Aulia Reservoir there are other Fassiekh production areas in the White Nile, further south at the riverine towns of Duiem, Kosti, Jabalain and Renk Yousif, 1988).Microbial spoilage

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and deterioration was observed in Fassiekh produced in Sudan. This was attributed to the use of bruised fresh fish, insufficient salting during curing as well as improper handling.

The addition of chemicals or other adjuncts to improve the keeping quality or consumer's appeal of food in general and fish products in particular are accepted practices. The traditional preservatives including salt, vinegar and acetic acid, alcohol and natural smoke are universally acceptable, and very few artificial preservative (e.g., sulphur dioxide, sorbic and benzoic acids), are permitted in fish products Connell, (1995).

Plant products, particularly spices and extracts of various plant parts have been used extensively as natural antimicrobials and antioxidants. In the commercial preservation of fish and fish products, natural antioxidants from plant sources have been found to extend shelf life and prevent fishy taste and flavor George *et al*., (2009).

Fans of hot, spicy cuisine can inhibit nasty bacteria and other food-borne pathogens. The recipes that come from countries with hot climates, humans' use of antimicrobial spices developed in parallel with food-spoilage microorganisms. Capsicums, including chilies and other hot peppers, are in the middle of the antimicrobial pack (killing or inhibiting up to 75 percent of bacteria; Sherman, 1998).

Ethanol extracts of the fruits of three kinds of Capsicum showed similar potencies in their antimicrobial activities against gram (+ve) and gram (-ve) bacteria, and fungi, although they contained different levels of capsaicin (citation Soetarno *et al.,* 1997). Bioautographic tests demonstrated that capsaicin was the main antimicrobial component Soetarno *et al.,* 1997). These results suggest that all kinds of Capsicum fruits tested are useful as antibacterial and anticandidal agents and not necessarily the most pungent pepper as in the traditional use (Djarwaningsih, 1992; Syamsuhidayat and Hutapea, 1991). Pepper contains high amounts of vitamin C and carotene (provitamin A; good source of most B vitamins, vitamin B6,high in potassium, magnesium and iron in addition to its vitamin C content which can substantially increase the uptake of non-heme iron from other ingredients in a meal, such as beans and grains (Paul and Deborah, 1980).

The main objectives of this study were to evaluate and to determine the efficiency of two types of pepper (hot and sweet), as natural antimicrobial and studying the possibility of employing them in preservation of Fassiekh product in Sudan where spoilage is caused mainly by microbial activity and certain type of spoilage microorganism.

MATERIALS AND METHODS

Experimental Trials

Fassiekh product and two different pepper fruits (fruit-1 *Capsicum annuum*-sweet pepper and fruit-2 *Capsicum frutescens* -hot pepper) were purchased from the local market of Khartoum-Sudan.two pepper fruits were transferred to Khartoum University, Faculty of Agriculture for taxonomic identification. The pepper fruits were dried and ground into powder using hand grinder. Fassiekh product made from (*Hydrocynus spp*.) fish was minced into homogenous mixture and divided into 3 groups (raw Fassiekh as control; Fassiekh mixed with sweet pepper and Fassiekh mixed with hot pepper). Each of three groups was divided into 4 sub-groups to finalize the desirable experimental sample. Random sample from each sub group was taken and transferred to Customs laboratory, SSMO (Sudanese Standard and Metrology Organization), and Laboratory of the State Ministry of Health / (Port-Sudan) for proximate analysis (moisture, protein, fat and ash) using AOAC methods AOAC, (1980).

pH measurement

One gram of homogenized Fessiekh from each sample was added to 10 ml of distilled water into a test tube. The pH level was determined using pH meter instrument model JENNAY 3015.

Microbiological investigation:

The microbiological test was conducted for total bacterial count (TBC) and identification test of *Staphylococcus aureus* and *Listeria monocytogens.* Minced Fassiekh (25 g) was added to warm sterile Peptone water (37^{o}C; 225 ml)and was shaken well to distribute all the organisms within test tube and 10 ml from the previous mixture was transferred with a 10 ml-sterile graduated pipette to Peptone water (90 ml) and mixed thoroughly. Using another sterile pipette, 2 ml of the dilution prepared was transferred to another bottle containing 18 ml of sterile Peptone water.

Total Bacterial counts

A sterile pipette was used to transfer 1ml of a selected dilution into duplicate sterile plates that contained nutrient agar the plates were incubated at 37^{o}C for 24 hours. Colonies were counted on the opposite side of the plate on its position on colonies counter apparatus.

Enumeration of coagulase positive Staphylococcus aureus

Suitable decimal dilutions of the sample prepared above were used. Pre dried Baird Parker Medium was inoculated with a total volume of 1.0ml by spreading 0.4, 0.3 and 0.3 ml into 3 plates. The inoculated media was incubated at 35°C ± 1°C for 45 - 48 hours. The plates were examined for typical *S. aureus* colonies which were grey to black, smooth, shiny, and convex, with an off white edge which may show an opaque zone and/or a clear halo extending beyond an opaque zone. The number of typical colonies were counted and recorded for each plate. Typical colonies were selected at random, counted and subcultured into Brain Heart Infusion broth, then incubated at 35°C for 18-24 hours and the ability to produce coagulase was tested

Coagulase test:

0.1ml of each Brain Heart Infusion (BHI) broth culture was added to 0.5ml of plasma and incubated at 35°C. The tubes were periodically examined over 6 hours for coagulation.

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Microscopic Examination:

A gram stain of *S. aureus* cultures produced gram positive cocci, 0.8 to 1.0 um in diameter occurring singly, in pairs or most frequently in irregular clusters resembling clusters of grapes.

Catalase Test:

Plates were flooded with 3.0% hydrogen peroxide solution or a loop full of colony was transferred to a slide and mixed with 3% hydrogen peroxide. Bubble formation was observed for colonies exhibiting no evidence of gas formation were catalase negative.

Isolation and detection of Lisrteria sp:

Detection of *Listeria monocytogenes* relies on enrichment and selective enrichment procedures, followed by isolation using selective plating techniques, with confirmation by biochemical and serological methods.

Primary enrichment:

Primary enrichment broth contains nalidixic acid and acriflavine for selectivity. 25 ml or g. sample were added to 225 ml of Lister Enrichment Broth (LEB) medium and UVM I Formulation and was incubated for 24 hours at 30°C.

Secondary enrichment:

This broth is identical to the primary enrichment broth except for increased acriflavin content to aid in selection and the addition of Lithium chloride and ferric ammonium citrate to produce visual blackening of tubes containing esculin-hydrolyzing bacteria. 0.1 ml of incubated, broth was transferred to Fraser broth. The inoculated medium was incubated at 35°C.

Isolation

Incubated broth was streaked on selective media containing cycloheximide, acriflavine, colistin sulphate, cefotetan, fosfomycin, polymixin B, acriflavin hydrochloride and ftazidime. Typical colonies were picked up for identification by biochemical and serological procedures. One loop full from incubated Fraser Broth was streaked onto *Listeria* Selective agar and/or PALCAM agar (PALCAM Agar base (7669) was used with supplements as selective and differential medium for detection and isolation of Listeria monocytogenes from food and environmental samples) a so that well isolated colonies could be obtained. The plate then incubated at 35°C for 24 hours. Typical *Listeria* colonies were surrounded by black halo and with a black sunken centre on *Listeria* selective agar. On PALCAM agar typical *Listeria spp*. form colonies that were app. 2 mm in diameter grey-green in color with a black sunken centre and black halo against a cherry red medium background.

Statistical analysis

The data obtained were analyzed using SPSS software (Version 10), one way ANOVA test as described by Gomez and Gomez 1984.

RESULTS AND DISSCUTION

The aim of this study was to determine and evaluate the efficiency of pepper fruits on the microorganisms of spoilage on Fessiekh. The findings of this research are presented in tables (1-4) and figures (1, 2, 3 and 4). The pepper became accepted on five continents as a healing agent as well as a seasoning. It is not only a folk remedy; although limited, its medical value has been proven scientifically. The anti-microbial properties of the spice capsicum are said to be inconsequential seemingly contradictory findings resulted in a 1993 study by researchers at Louisiana state university Medical Centre in New Orleans, who found that straight hot pepper sauce killed all the bacteria in a test tube within a minute, (Andrwes, 1995).

The findings of this study were in agreement with many authors such as Hussien, (2002) and Andrwes (1995), who reported a moisture content range (74.94-60.20%), for dry salted *Hydrocynus forskalii*. The protein results were slightly lower than protein range of (18.4 -71.9%) reported for fermented fish in Africa by FAO (1992).The protein content of studied "Fassiekh" showed no systematic variation in protein content after adding of pepper. Fat content was in agreement with the range (6.68±2.26% wet weight (W) which was reported by Omer, (1984) for dry salted *Hydrocynus spp* and slightly higher than values of (1.48-5.97%) recorded by Hussien, (2002).

Ash results were in agreement with those reported by Mahmoud (1977), who found an average ash content of (16.61±2.26% D.W) and (15.87±6.32% D.W), reported by (Omer, 1984) for dry salted *Hydrocynus spp.* Results found indicate that there was no systematic variation in chemical composition of studied Fessiekh product after treatment with the two types of hot and sweet pepper. This might be due to the high variation on its moisture content.

From the results, the total viable count of bacteria in the studied Fassiekh product of *Hydrocynus spp* as raw material was $43.4x10^3 \pm 1.3x10^3$ CFU, and these viable bacteria counts were higher when compared to the laboratory prepared Fassiekh sample. This could be explained by the quality of fish, used for Fassiekh production and amount of salts which were added and techniques used in commercial production. Eltom, (1989) reported that after the addition of the salt in Fassiekh fermentation, the viable count rose to 1.8×10^8 cell/g on the fourth day, then after it, were decreased steadily, to 8.6×10^5 cell/g on day twelve. This pattern of rise and fall in the microbial count was observed during fish fermentation in Fassiekh of Egypt Hamed et al., (1973) and in the fish fermentation of south East Asia Saisithi *et al.*, (1967). Fassiekh of *Hydrocynus spp.* with sweet pepper performed higher range of viable counts of bacteria $64.5 \times 10^3 \pm 4 \times 10^3$ CFU than *Hydrocynus spp* with hot pepper $4.5 \times 10^3 \pm 1 \times 10^3$ cfu after 96 hrs, this may be due to the amount of capsaicin in *Capsicums* which varies and dependent on genetics, giving almost all types of capsicums varied amounts of perceived heat. The dominated bacterial genera isolated from Fassiekh (*Hydrocynus spp*) studied was *Staphylococcus sp*. with absence of *S. aureus*. It seems that this group is more salt resistant than the others existing in the fish. El tom,

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(1989) found that the most commonly encountered bacterial genera in Fassiekh fermentation were *Bacillus*, *Staphylococcus* and *Micrococcus.* The presence of *S. aureus* in dried foods indicates contamination from the skin, mouth or nose of food handlers FAO, (1992).

The present study indicated that the bacterial genus (*Staphylococcus sp*.) was isolated from cured Fassiekh and Fassiekh treated with sweet pepper. While was not found on Fassiekh that treated with hot pepper. Also the genus *Listeria sp.* was isolated from Fassiekh treated with sweet and hot pepper, and was not observed in cured Fassiekh. The *Listeria monocytogens* and *Staphylococcus aureus* tests were negative for all samples.

It could be concluded that, the pepper fruits can play as antimicrobial agent in conserving the Fassiekh product by lowering the total viable count, killing or inhibiting some organism that related to Fassiekh spoilage, and recommend that, the hot pepper can be used in small amounts in preparation and production of Fassiekh.

Table 1. The effect of pepper spices on the chemical composition of Fassiekh product.

Parameter / Treatment	Moisture %	Protein %	Ash %	Fat %	pH level
Raw Fassiekh	56.7 ± 5.2**	12.3±0.86	17.4±0.20*	6.9±0.30	6.8±0.11*
Fassiekh +5% Sweet pepper	65.1 ± 4.4**	12.5±1.04	17.6±0.15*	6.9±0.25	6.7±1.26*
Fassiekh + 5% Hot pepper	61.2 ± 2.8**	12.6±1.03	17.7±1.25 *	6.9 ± .24	6.7±0.11*

** = Highly significant differences ($p<0.001$). * = Significant differences ($p<0.05$)

Table 2. Indicates the microbial load on the Fassiekh products during different interval periods

Time / Treatment	0 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.
Fassiekh+5% Sweet pepper	43.4×10^3 NS $\pm 1.3\times10^3$	$113.4\times10^{3**} \pm 4.5\times10^3$	$84\times10^{3**} \pm 10.6\times10^3$	$65\times10^{3**} \pm 4.9\times10^3$	$64.5\times10^{**3} \pm 4\times10^3$
Fassiekh+5% Hot pepper	43.4×10^3 NS $\pm 1.3\times10^3$	$47\times10^{3**} \pm 3.5\times10^3$	$32.4\times10^{3**} \pm 6.4\times10^3$	$6\times10^{3**} \pm 1.6\times10^3$	$4.5\times10^{3**} \pm 1\times10^3$

** = Highly significant differences ($p<0.001$). NS= No significant differences

Table 3. Indicates the microbial load on studied Fassiekh products

Parameter / Treatment	Staph. Sp.	Staphylococcus aureus	Listeria Sp.	Listeria monocytogens
Raw Fassiekh	+Ve	Nil	Nil	Nil
Fassiekh+5% Sweet pepper	+Ve	Nil	+Ve	Nil
Fassiekh+5% Hot pepper	Nil	Nil	+Ve	Nil

Table 4. Effect of sweet pepper and hot pepper on the *Staphylococcus* count after 96 hrs

Parameter / Treatment	Staph	*Staphylococcus aureus*
Raw Fassiekh	$7.6x10^3$	-ve
Fassiekh + 5% Sweet pepper	$21.9x10^3$	-ve

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Fassiekh + 5% Hot pepper	-ve	-ve

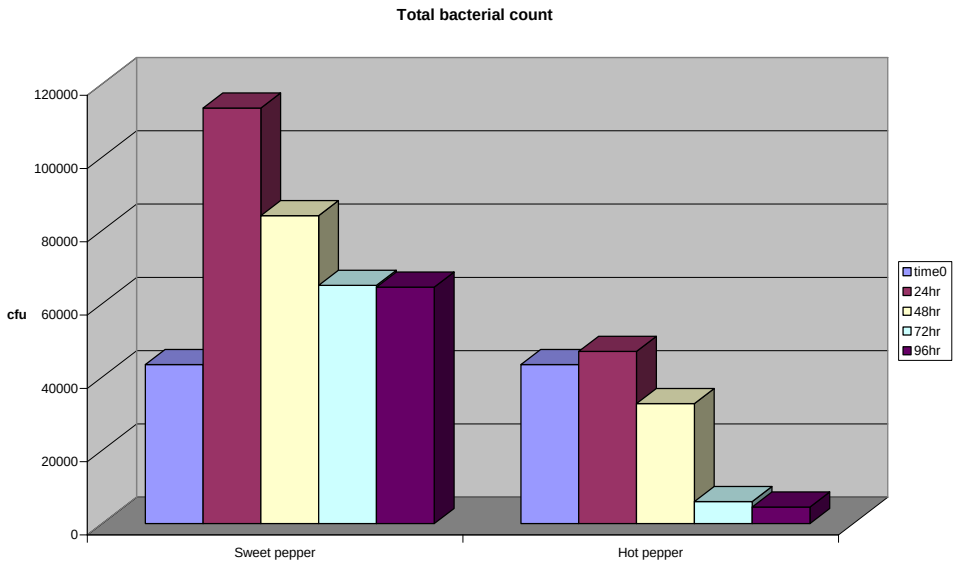

Figure 1. Effects of the total bacterial count on the Fassiekh treated with sweet and hot pepper during different interval periods

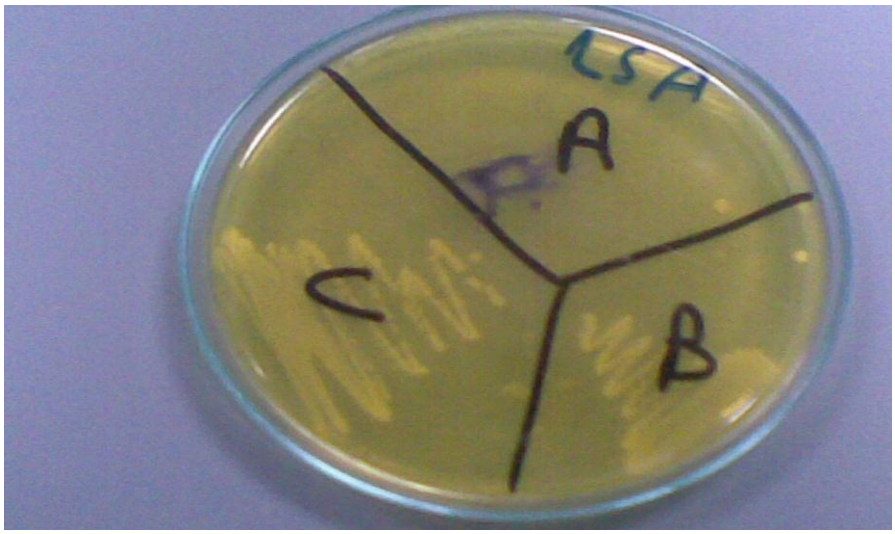

Figure 2. Fassiekh sample on Listeria Selective agar Where A: Cured Fassiekh (–ve); B: Fassiekh +sweet pepper (+ve); C: Fassiekh +hot pepper (+ve)

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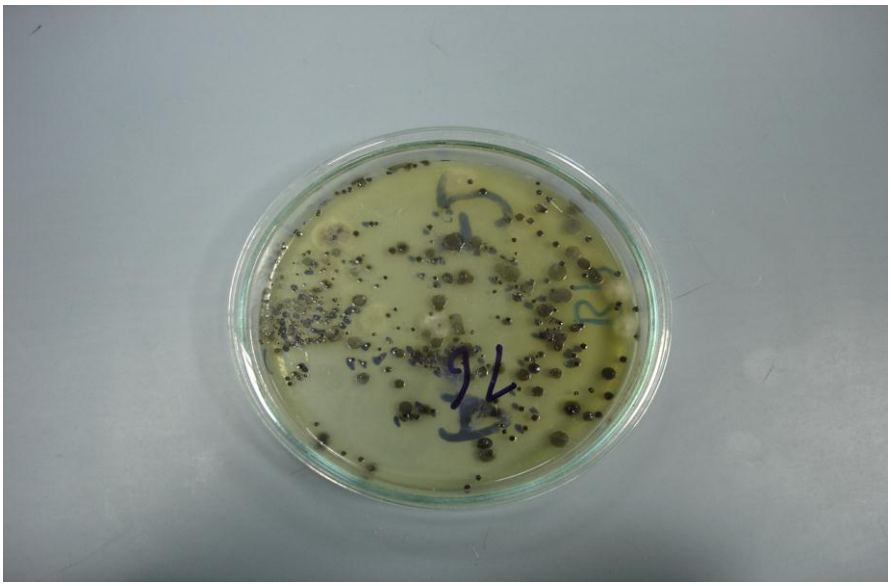

Figure 3. Crude Fassiekh sample on Bared barker agar for staph test

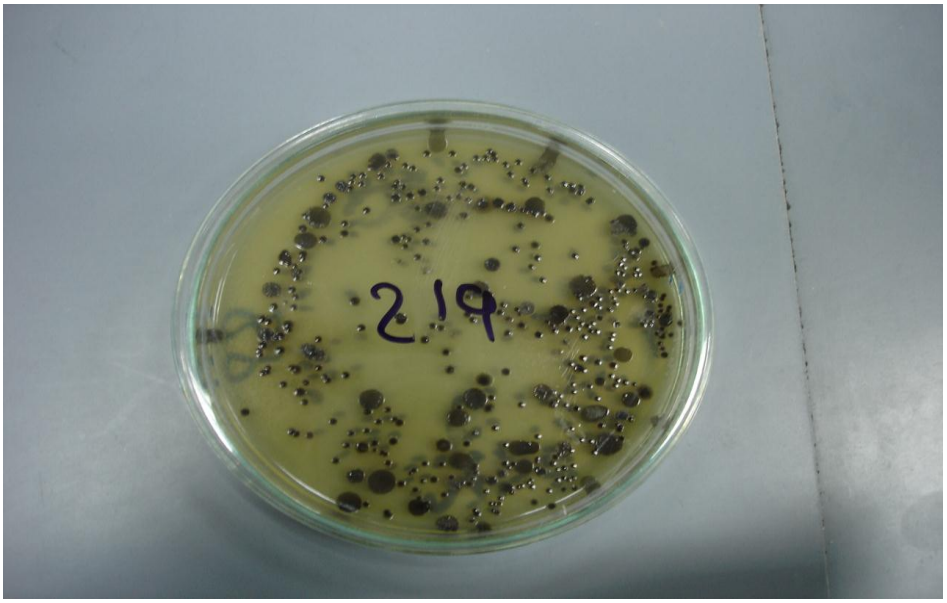

Figure4. Fassiekh + Sweet pepper sample on Bared barker agar for staph test

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