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**The effects of lemon Juice and acetic acid at
different levels on total bacterial count from
beef Minced Meat**

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

نَرْفَعُ دَرَجَاتٍ مِّنْ نَّشَأٍ ۗ

وَفَوْقَ

كُلِّ ذِي عِلْمٍ عَظِيمٍ

صدق الله العظيم

سورة يوسف: 76

الإهداء

إلى بحر العجب والحنان والفيض الساكن في عروقي
التي لم تنم يوماً إلا ورفعت يدها للسماء تدعو الله ليحقق

حلمي

أمي الحنوننة...

إلى الذي أوصاني الله به برأ وإحساناً وأهدى لي سنين

عمري

الذي أحنى ظهره التعب في سبيل وصولي لهذه

المرحلة

والذي الحبيب...

إلى نجوم سمائي المتلألئة وسندي في الحياة

إخوتي...

إلى سندي المستقبل وشمعة العطاء وأمل الغد المشرق

أساتذتي...

أهدي اليهم ثمرة جهدي المتواضع هذا ...

شكراً لكل من وقف في جانبي وساندني في تحقيق

هذا النجاح..

شكر وتقدير

ممما حاولنا جاهدين أن نعبر لكل من وقف بجانبنا وزادنا إصراراً لاستكمال مسيرتنا، وممما أخبرناهم عن ما في قلوبنا من شكر وعرفان لإيمانهم بقدرتنا عند المصاعب التي واجهتنا، فالكلمات والحروف لن توفي قدرهم ولا صبرهم علينا، لهذا نقدم لكم هنا أجمل العبارات عن الشكر والعرفان لعلها تعبر ولو قليلاً عما نشعر به:

. أستاذتي الكرام .. كل التبريل والتوقير لكم، يا من صنعتم لي المجد، بفضلكم فهمت معنى الحياة، اسقيت منكم العلوم والمعارف والتجارب واخص بالذكر الدكتور الفاضل (رعد جبار حمودي) الذي ساندني ووقف بجانبني وقدم لي كل شيء من اجل اتمام بحثي
هنا

. أصدقائي الرائعون .. رسالة شكر وعرفان أطيرها لكم لوقوفكم بجانبني دوماً، فلو تحبتم عن ناظري يوماً فأنتم في القلب، أذكر أيام الشدائد حينما لم تفارقوني لحظة، بل كنتم خير عون، وسند، وناصح، ما أجمل تلك الأيام بكل ما فيها، فلقد كنتم كالسكر الذي يذهب مرارة العيش، ويسلي النفس، ويشد من أزرها، سعادتي كبيرة بكم، ولن أتخطى عنكم ما حييت. وأخص بالذكر الى اخواتي وصديقاتي
(وسن وزينب)

. أهدي لكم ثمرة جهدي هذا

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Abstract

Purpose – of this study is to investigate the effect of lemon juice and acetic acid at different levels on total bacterial count from minced beef meal.

Design – Methodology / approach – beef minced meat were inoculated one by one both bacteria at high inoculum levels and were treated with different concentration fresh lemon juice and acetic acid (2.5% , 5% , 7.5% , 10%) for (3hr,6hr,12hr,24hr)

Findings – addition of lemon Juice different exposure time and different concentration cause reduction between and different 41.3 and 19.8 log CFu/ml for total bacteria count while 19.6 and 12 when added the acetic acid in different concentration and time result showed that lemon Juice and acetic acid. Slight decrease as an immediate inhibitor but this effect increase with concentration and time result showed that acetic acid caused decrease in total bacterial count more than lemon juice when comparative together.

Originality / Value – this is research study to provide information on the effective before cooking and eating of minced meat on the presence of the bacterial count and to strengthen the hygienic quality of beef minced meat . Inactivation of lemon juice and acetic acid on total bacterial count may give practical and easy way of providing food safety for minced meat.

1- Introduction

Lemon juice and acetic acid were investigated for their antimicrobial. Numerous food products require protection from spoilage during preparation, storage, and distribution to gain them the desired shelf life. The minimally processed, easily prepared and ready-to-eat fresh food products, globalization of food trade and distribution from centralized processing pose major challenges for food safety and quality [1].

The quality of food and food products may suffer harmful damaging during their preparation, storage, and distribution by microbiological and/or chemical changes that seem to be a major factor affecting their quality, safety and cost. Microbial contamination can cause major public health hazards and economic loss in terms of food poisoning and meat spoilage (2).

Thus, the application of suitable agents possessing both antioxidant and antimicrobial activities may be useful for maintaining meat quality, extending shelf-life and preventing economic loss(3).

Numerous efforts are conducted to find natural alternatives to prevent bacterial and fungal growth as well to inhibit the oxidation process in foods .Many researchers have indicated that lipid oxidation and microbial growth in meat products(e.g. plant and/or herbs) possessing antioxidant have the advantage of being readily accepted by consumers (2).

Screening of plant extracts revealed that majority of the plant extracts contains phenolic compounds as secondary metabolites. Biological significance of these compounds is immense due to enormous reducing power of free hydroxyl groups (antioxidant property) and protein binding capacity (causes inhibition of microbial growth–antimicrobial property) (4).

It is worthy to note that the demand for medicinal herb (i.e. plants contain phytochemicals) has begun to grow and gain popularity. (5).

reported that some of the selected medicinal herbs of food ingredients (e.g. coriander, ginger, lemongrass, and sweet basil,...etc) had antimicrobial activity. There are conflicting reports as to whether there is greater bacterial inhibition by acetic compared to lactic or citric acid washes. Lactic

acid (2%) was shown to reduce *E. coli* O157:H7 on beef carcass tissue by 3.3 log, and 2% acetic acid reduced it by 1.6 log (6).

These authors also found that lactic acid and acetic acid treatments on cheek meat, using spray or immersion, resulted in 1.1 log reductions in total bacteria. The mechanism of action of organic acids on the microbial cell is not completely understood, but it is hypothesised that it is the undissociated molecule of the acid that is responsible for the antimicrobial activity. There is a lot of variability in the literature in terms of the cited reductions that can be achieved. This is mainly due to differences in the concentrations of the acids used by different researchers, the method of application, and the types of samples tested. There is also some evidence that organic acids may enhance the shelf life of modified atmosphere packaged product, mainly because they increase the lag phase of the microorganisms (7). Acetic acid, a food acidulant is often used in meat marination to improve the water holding capacity (WHC) and tenderness of beef muscle (8).

The mechanism of the tenderising action of acidic marinades is believed to involve in the weakening of structures due to swelling of the meat and increased conversion of collagen to gelatin at low pH during cooking (9). Therefore, the objective of this study was to compare the effects of acetic acid marinate on the sensory attributes such as marination mass gain, cooking loss, tenderness, colour and flavor of meat from culling cows. Accordingly, the objectives of this study were intended to investigate the antimicrobial and the activities of lemon juice and acetic acid set up the optimum agents to be added to minced beef meat in order to reduce oxidative and microbiological deterioration. Lemon juice and acetic acid at the optimum concentrations on some quality parameters e.g pH values. Microbiological counts of the prepared minced beef meat.

2-Material and method

Material	Method
Refrigerator	japan
Sensitivebalance	China
Incubator	China
Sterilemagnetic	japan
Micropipette	Japan
Petri dish	India

2-1 culture media

Nutrient agar[HI media India]

2-2 Minced beef meat

The minced beef meat were obtained from local butcher shops . The animal were slaughtered, dressed and de-boned manually and mincing and packed in polyethylene bags and stored overnight at $4 \pm 1^{\circ}\text{C}$ in a refrigerator.

2-3 material:

Lemon fruit I were used in this study as sources of antioxidant and antimicrobial agents. Fresh lemon juice and were purchased from a local market. And acetic acid were at from chemical laboratory in medicine college in diyala university

2 -4 Preparation of plant sample {lemon and acetic acid}

Lemon juice were taken off from the fruits. For preparation of lemon juice were cut into half and squeezed by a hand-pressed juice extractor [10] and also ph value ph mater [HAANA , HI 902 METER ,JERMANY]. Lemon fruit and acetic acid were kept in closed containers in a refrigerator at 4°C until

2-5 required Preparation of minced beef meat:

The minced beef meat was subdivided into four for 25g. Minced beef meat were prepared to provide four concentration [2.5% ; 5 %; 7.5% ;10%] of lemon juice and acetic acid . A control was formulated without lemon juice and acetic acid . The other treatments were prepared by adding the optimum concentrations determined of the tested addition to the minced beef meat as follows[2.5% ,5% ,7.5% ,10%] lemon juice and [2.5% ,5% ,7.5% ,10%] acetic acid then soaking at different time [3hr ,6hr,12hr,24hr] meat former. Minced beef meat were placed on plastic foam meat trays, wrapped with polyethylene film and kept in a refrigerator at 4°C . The effect of the optimum concentration of the test addition of the lemon juice and acetic acid , pH & stander plate count (SPC) were determined in minced beef meat during the storage time at 4 °C.

2-6 stander plate count (SPC):

The stander plate count was determined on nutrient agar medium as recommended by the American Public Health Association for food stuff examination (APHA 1992). Plates seeded with serial dilutions [10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , 10^{-10} , 10^{-11}] of the samples were incubated at 37°C for 24-48 h

2-7 Inoculation of minced beef meat dilution with nutrieni agar

25g of minced beef meat was added in 225ml of distal water and manual method . Then, we taken 0.1 m from serial dilution was [10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , 10^{-10} , 10^{-11}]transferred to plate count agar (HI media india); pour plate incubated at 37C for 24h. The initial bacterial population was appear and count colony

2-8 Ph determination

A minced beef count (25 g) was soaking in the lemon juice [3hr ,6hr , 12hr , 24hr] and the pH was measured using a digital pH-meter (HAANA, HI902 meter, Germany).Three readings were taken from each of the four minced beef count

3–results

The PH of lemon Juice was 2.54 the initial PH of Minced beef Meat 5.8 . When it was added with lemon Juice the average PH of the Mixture became to 5.3 and decrease 4.6 at the end of the experiments while addition of acetic acid the average PH of the mixture became to 4.3 and decrease to 3.8 at the end of the experiments .The table 1.2 show the effects of different and concentration of lemon Juice (2.5%, 5% , 7.5% and 10%) on total bacterial count inoculated to beef Minced Meat at different exposure time (3hr , 6hr , 12hr , 24hr) .When minced beef meat minced beef sample were soaking with different concentration of lemon Juice the reduction on the initial total bacterial count in each group average from 41.3 to 36.4 log cfu/MI 35.4 to 25.6 log CFu/MI 25.5 to 22.1 log CFu/MI 21.3 to 19.8 log CFu/MI respectively at different exposure time .The table 3.4 show the effects of different concentration of acetic acid (2.5%, 5%, 7.5% and 10%) on total bacterial count inoculated to beef Minced Meat at different exposure time (3hr , 6hr, 12 hr, 24hr) when Minced beef Meat sample were soaking with different .Concentration of acetic the reduction on the initial total bacterial count in each group average from 19.6 to 19 log cfu/MI, 18.2 to 17.5 log cfu/MI , 17.4 to 15.8 log cfu/MI , 14.1 to 12 log cfu/MI respectively at different exposure time

Table (1) of ph & bacterial count in different concentration & time in lemon juice

Concentration 2.5%	Time	ph	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	Average of spc cfu/ml
	3hr	5.5	60	58	48	41	46	37	32	26	24	41.3
	6hr	5.4	35	35	37	32	35	40	36	40	49	37.6
	12hr	5.3	50	48	48	44	36	35	30	25	19	37.2
	24hr	5.25	29	31	32	36	39	38	40	24	21	36.4

Concentration 5%	Time	ph	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	Average of spc cfu/ml
	3hr	5.20	48	35	23	32	23	38	45	40	35	35.4
	6hr	5.15	32	18	19	51	40	32	22	26	20	28.8
	12hr	5.14	35	40	18	35	25	20	15	22	25	26.1
	24hr	5.12	33	22	23	28	18	33	25	33	16	25.6

Table (2) of ph & bacterial count in different concentration & time in lemon juice

Concentration 7.5%	Time	ph	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	Average of spc cfu/ml
	3hr	4.95	35	31	28	20	22	30	19	25	20	25.5
	6hr	4.88	18	30	35	28	18	20	32	30	16	25.2
	12hr	4.86	31	22	24	21	25	15	14	29	25	22.8
	24hr	4.83	23	20	20	16	29	21	23	26	21	22.1

Concentration 10%	Time	ph	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	Average of spc cfu/ml
	3hr	4.81	30	23	22	25	22	19	18	17	16	21.3
	6hr	4.74	32	22	18	11	18	21	22	21	27	21.3
	12hr	4.73	37	30	21	12	16	14	12	12	26	20
	24hr	4.45	13	39	18	10	16	13	21	28	21	19.8

Table (3) of ph & bacterial count in different concentration & time in acetic acid

Concentration 2.5%	Time	ph	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	Average of spc cfu/ml
	3hr	4.41	29	21	14	13	17	26	22	15	19	19.6
	6hr	4.38	30	20	22	16	10	16	27	18	15	19.3
	12hr	4.29	22	19	22	18	19	15	22	16	20	19.2
	24hr	4.24	12	20	28	25	20	27	11	12	17	19

Concentration 5%	Time	ph	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	Average of spc cfu/ml
	3hr	4.10	27	30	35	20	18	20	16	18	15	18.2
	6hr	4.1	16	15	14	20	12	14	18	22	30	17.8
	12hr	4.05	14	16	12	18	20	18	15	19	27	17.6
	24hr	4.014	17	12	23	25	16	20	14	16	15	17.5

Table (4) of ph & bacterial count in different concentration & time in acetic acid

Concentration 7.5%	Time	ph	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	Average of spc cfu/ml
	3hr	4.008	18	15	25	18	22	24	20	15	12	17.4
	6hr	3.39	12	14	10	12	16	15	28	20	27	17.1
	12hr	3.91	23	14	15	12	16	30	12	15	12	16.5
	24hr	3.90	20	19	10	18	13	10	12	20	21	15.8

Concentration 10%	Time	ph	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	Average of spc cfu/ml
	3hr	3.9	17	15	13	13	12	16	15	12	15	14.1
	6hr	3.86	11	17	15	12	13	14	14	16	12	13.7
	12hr	3.8	11	13	10	14	13	15	12	14	10	12.4
	24hr	3.79	10	15	9	10	18	13	12	11	10	12

4-Discussion

The presence of weak acid at different concentration in household products such as lemon juice and lemon dressing led to hypo this is that the product could be used as sanitizers in home use of food like raw products(10).

These natural product and their mixture can be considered to be potential antimicrobial agent in preventing food borne out breaks related to fresh products at house hold levels (11) however soaking of minced beef meat with lemon juice and acetic acid for 24 hr not enough to prevent contamination with different bacteria but at enough to reduce the microbial count in row meat. Inactivation effect lemon juice and active acid give a practical and easy way of providing food safety for beef minced meat consumers generally prefer to eat raw meat just after squeezing fresh lemon juice or added acetic acid and these could be away to minimize the amount of existing these could be away to minimize the amount of existing microorganism results of this study showed that lemon juice caused slight decrease of microorganism are immediate inhibitor but this effect increased by concentration and time generally appear in this study that the activation effect of acetic acid for inhibitor and reduce of microorganism is more then lemon juice and also this effect increase by concentration and time (6)

الملخص/

الغرض من هذه الدراسة هو التحري عن تأثير عصير الليمون وحامض ألكليك في مستويات مختلفة على العدد الكلي للبكتيرية في اللحم البقري المفروم .

تصميم -طرق عملية التجربة -هو معالجة اللحم البقري المفروم بتركيز مختلفة من عصير الليمون الطازج وحامض ألكليك (25%، 5%، 7، 5%، 10%) وبأوقات مختلفة (3 ساعات ، 6 ساعات 12 ساعة 24 ساعة) لكل التركيز على التوالي

النتائج -إضافة عصير الليمون الطازج الى اللحم المفروم ولأوقات مختلفة وتركيز مختلفة أدى إلى تقليل العدد الكلي البكتيري ما بين (19.8 إلى 41.3) $\log_{10} \text{CFU/MI}$ بينما وجده عنده إضافة

حامض ألكليك ولأوقات مختلفة إلى عينات اللحم المفروم أدى إلى تقليل العدد الكلي البكتيري

ما بين (K/19.6) $\log_{10} \text{CUF/MI}$. وبشكل عام وجده عنده إضافة كل من عصير الليمون وحامض ألكليك يظهر انخفاض طفيف وبشكل سريع في تنشيط النمو البكتيري ولكن هذا التأثير يزداد بزيادة التركيز والوقت - وبشكل عام أظهرت النتائج بان حامض ألكليك يؤدي الى انخفاض في العد الكلي للبكتيرية أكثر من عصير الليمون عنده المقارنة

الهدف من البحث -هو توفير المعلومات حول فعالية الإضافات الغذائية او النكهات قبل الطبخ والأكل واللحم المفروم من حيث وجود العد البكتيري وكذلك لتعزيز الجودة الصحية للحم البقري المفروم وكذلك ملاحظة فعالية كل من الليمون وحامض ألكليك على العدد الكلي للبكتيريا قد تعطي طرق علمية وسهلة لتوفير سلامة الأغذية عنده تناول اللحم المفروم .

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