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Effects of lemon and kiwi juice in reduction of some pathogens contaminating chicken breast meat

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ABSTRACT

The unacceptable bad quality chickens meat is a problem for the poultry industry. The main objective of the current study was to improve the sensory and microbiological quality of raw chicken meat using natural juices of kiwi, lemon, and their combination, and to investigate the deterioration criteria and sensory quality of treated meat during refrigerated storage. Meat were marinated in marinade solutions containing fruit juices in five groups as follow: kiwi juice (5%), kiwi juice (10%), lemon juice (0.04%), lemon juice (0.2%), mixture of kiwi (10%) and lemon (0.2%) juices and a control group without marination. All groups were stored at 4 °C. Results revealed that the reduction % in APC was 73.9%, 89.3%, 95.3%, 98% and 93.3% and in Enterobacteriacae count was 89.23%, 95.64%, 58%, 99.8% and 95.51%, and in Staphylococci count was 60.60%, 93.18%, 65.15%, 96.74% and 93.68 in kiwi (5%), kiwi (10%), lemon (0,04%), lemon (0.2%), and mixture of lemon (0.2%) and kiwi (10%), respectively**.** So, these natural juices can be used as natural preservatives to control bacterial contamination and increase shelf life of chicken meat.

1. INTRODUCTION

Chicken meat is an animal product that is important for human nutrition, has a variable and moderate energy content, highly digestible proteins of good nutritional quality, unsaturated lipids, fat-soluble and B-complex vitamins as well as minerals that make poultry meat a valuable food. (Donma et al.,2017).

Chicken meat is the ideal medium for bacterial growth due to its high moisture content, richness in nitrogenous compounds (essential amino acids, proteins), a good source of minerals, vitamins, and other growth factors. However, its PH is favorable to the growth of microorganisms.

The bacteriological safety of food continues to be a major concern for consumers, regulatory agencies, and the food industry.

Traditionally, many food preservation strategies have been used to control microbial spoilage in food, but contamination of food and spoilage by microorganisms is still a problem that needs to be properly controlled. (Christensen et al., 2009)

Although synthetic antimicrobials have been approved in many countries, recent trends in the use of natural preservatives have necessitated exploration of alternative sources of safe, effective, and acceptable natural preservatives (Sullivan and Calkins,2010)

Plants contain innumerable constituents and valuable sources of new and biologically active molecules with antimicrobial properties and proteolytic enzymes that affect the tenderness of the meat (Ketnawa and Rawdkuen,2011).

Kiwi fruit extract has an antibacterial effect against some gram-negative and gram-positive bacteria and plays an effective role in meat tenderization (Ketnawa and Rawdkuen,2011). Kiwi fruits have protease enzyme (actinidin) that is very effective in meat tenderization and has antifungal, antioxidant, and antibacterial effect on meat (Koak et al., 2011)

Lemon is an important medicinal plant in the Rutaceae family. It is a rich source of vitamin C and is cultivated mainly for its alkaloids, which have anticancer activity and antibacterial potential in crude extracts of different parts (viz., leaves, stem, root, and flower).

Citrus flavonoids have a wide range of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities (Ke et al., 2009)

So, the main objective of the current study was to investigate the effects of kiwi and lemon juices marinade in various concentrations on the Bacterial contamination of chicken meat in refrigerated storage.

2. MATERIAL AND METHODS

1.1. Samples:

Three Kg. of chicken breast fillets were purchased from local markets in Tanta, El-Gharbia governorate, Egypt and transferred to the lab and stored at 4 ±1°C.

1.2. Preparation of marinated solutions:

Fresh lemon and kiwi fruit were purchased from local supermarkets in Tanta, El-Gharbia governorate, Egypt, kiwi fruit was peeled, sliced, blended and filtered to obtain kiwi juice and lemon fruit was split, pressed and filtered to obtain lemon juice. Concentrations were kiwi juice(5%) , kiwi juice(10%), lemon juice(0.04%), lemon juice(0.2%),

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mixture of kiwi juice (10%)and lemon juice(0.2%) and a control group without margination.
The basic marinade solution was prepared using a simple traditional formulation as follows: salt 1.8 %, polyphosphates 0.3%, white pepper 0.002% (Abdelraman, 2016).

2.3. Experimental Design (*Abdelraman, 2016*)
Samples were divided into six groups (500 g of each) and marinated as following (1)
Marination was for 24hrs then , all groups were stored at 4 °C. Sodium tripolyphosphate, white peppers and sodium chloride were obtained from a local supermarket in local markets in Tanta, ElGharbia governorate, Egypt.
-A three replicate experiment was conducted.

2.4 Bacteriological analysis:
Samples were analyzed for detection of APC ,Enterobacteriaceae and Staphylococci counts at 0,3 , 6 and 9 days of storage(4°C) until appearance of spoilage signs.

2.5 Bacteriological investigation:
2.5.1. Sample preparation (ISO, 2017):
Twenty-five g. of chicken breast meat samples were transferred to a sterile stomach bag and 225 ml of 0.1% sterile peptone water was added aseptically to the contents of the bag. Each sample was then homogenized in the stomach (Biomreruxsa-France-no.42489367) at 2000 rpm for 1-2 minutes. One ml of homogenate was added to a sterile tube containing 9 ml of peptone water (0.1%) and then mixing. From which tenth – folded serial dilutions have been prepared.

2.5.2 Aerobic Plate Count (APC) was carried according to(ISO, 2013).

2.5.3. Enterobacteriaceae count (EBC) was counting on Violet red bile glucose agar (VRBG) according to(ISO,2004).

2.6.4. Staphylococci count was estimated on Baired Parker agar plate according to (ISO,1999).

2.7 Statistical analysis
Statistical data analysis for the three independent replicates was treated by one way ANOVA using SPSS program according to Feldman et al. (2003).

Table 1 Groups of samples (Abdelraman, 2016)

Groups	Description
1st	Control positive (chicken breast 500 g + marinade without kiwi or lemon
2nd	Kiwi 5% (chicken breast 500g + marinade containing kiwi 5%)
3rd	Kiwi 10% (chicken breast 500g + marinade containing kiwi 10%)
4th	Lemon 0.04%% (chicken breast 500g + marinade containing lemon 0.04%)
5th	Lemon 0.2%(chicken breast 500g + marinade containing lemon 0.2%)
6th	Mix (chicken breast 500g + marinade containing lemon 0.2% and kiwi 10%)

3. RESULTS

The results achieved in tables (2 and 3) it is evident that the initial mean count of total aerobes in untreated (control) group, was $3.30 \times 10^6 \pm 7.00 \times 10^5$ such count was slightly decreased to $8.60\times10^5\pm 2.60\times10^4$, $3.50\times10^5\pm 1.02\times10^4$, $1.53\times10^5\pm1.00\times10^5$, $5.00\times10^4\pm 2.20\times10^3$ and $2.20\times10^5\pm 1.00\times10^4$cfu/g after the treatment with kiwi 5%, kiwi 10%, lemon 0.04%, lemon 0.2% and mix (lemon 0.2% and kiwi 10%), respectively with reduction percentages of 73.9%, 89.3%, 95.3%, 98% and 93.3%, respectively.
By the 3rd day of the experiment, the mean count of total aerobes in control group was $5.10\times10^7\pm7.00\times10^6$ such count was slightly decreased to $9.5\times10^5\pm3.04\times10^4$, $1.50\times10^5\pm3.00\times10^4$, $3.12\times10^5\pm1.10\times10^4$, $1.50\times10^4\pm 1.00\times10^3$ and $1.20\times10^5\pm2.00\times10^4$ for the treated samples with kiwi 5%, kiwi 10%, lemon 0.04%, lemon 0.2% and mix (lemon 0.2% and kiwi 10%), respectively with reduction percentages of 71.2%, 95.4%, 90.5%, 99.5% and 96.3% respectively.
On the 6th day of refrigeration storage at (4±1ºC), the control samples, kiwi 5% and lemon 0.04% showed extreme discoloration and off-odor indicating a complete spoilage more than other treated samples and the mean counts were $5.00\times10^6\pm1.50\times10^4$, $7.00\times10^4\pm2.00\times10^3$ and $3.40\times10^5\pm2.00\times10^4$ log cfu/g for kiwi 10%, lemon 0.2% and mix (lemon 0.2% and kiwi 10%) , respectively with reduction percentages of -51%, 97.8% and -3%, respectively.
On the 9th day of refrigeration storage at (4±1ºC), the samples of control, kiwi 5%, kiwi 10%, lemon 0.04%and mix (lemon 0.2% and kiwi 10%) showed extreme discoloration and off-odour indicating a complete spoilage more than samples treated with lemon 0.2% and the mean count of them were $8.40\times10^4\pm 3.20\times10^3$ with reduction percentage was 97.4%.
From the results achieved in table (4 and 5) it is evident that the mean count of S. aureus in untreated (control) group, was $1.32X10^4 \pm 1.30X10^3$ log cfu/g such count was slightly decreased to $5.20X10^3 \pm 0.60X10^2$, $9.00\times10^2 \pm 4.30\times10^2$, $4.60\times10^3 \pm 1.20\times10^2$, $4.30\times10^2 \pm 0.30\times10^2$ and $8.33\times10^2 \pm 0.06\times10^2$ log cfu/g after the treatment with kiwi 5% ,kiwi 10%, lemon 0.04% ,lemon 0.2 and mix (lemon 0.2% and kiwi 10%), respectively in zero day with reduction percentages 60.60%, 93.18%, 65.15%, 96.74% and 93.68% respectively.
By the 3rd day of the experiment, the mean *S. aureus* count in control group was $4.00\times10^5 \pm 1.20\times10^4$ log cfu/g and the mean count was $2.03\times10^3 \pm 1.02\times10^2$, $3.1\times10^2 \pm 1.01\times10^2$, $1.00\times10^3 \pm 0.30\times10^3$, zero and $4.03X10^2 \pm 0.06X10^2$ log cfu/g for the treated samples with kiwi 5% ,kiwi 10% , lemon 0.04% ,lemon 0.2% and mix (lemon 0.2% and kiwi 10%), respectively, with reduction percentages 84.62%, 97.65%, 92.42%, 100% and 96.94%, respectively.
On the 6th day of refrigeration storage at (4±1 °C), the control samples, kiwi 5% and lemon0.04% showed extreme discoloration and off-odor indicating a complete spoilage more than other treated samples and the mean counts $2.03\times10^2\pm0.1\times10^2$, zero and $3.08\times10^2\pm2.3\times10^2$ log cfu/g for kiwi 10%, lemon 0.2% and mix (lemon 0.2% and kiwi 10%), respectively, with reduction percentages 98.6%, 100% and 97.66 %, respectively.
At the 9th day of refrigeration storage at (4±1ºC), the samples of control, kiwi 5%, kiwi 10%, lemon0.04% and mix (lemon 0.2% and kiwi 10%) showed extreme discoloration and off-odour indicating a complete spoilage more than samples treated with lemon 0.2%, the mean *S. aureus* counts were zero log cfu/g, with reduction percentages 100%.
From the results achieved in table (6 and 7) it is evident that the mean count of Enterobacteriacae in untreated (control) group, was $7.80\times10^4 \pm 1.06X10^3$ log cfu/g such count was slightly decreased to $3.40\times10^3 \pm 1.30X10^2$, $8.40\times10^3\pm 2.00X10^2$, $3.20\times10^4 \pm 0.03X10^3$, $1.00\times10^2 \pm 0.80X10^2$ and $3.50\times10^3 \pm 0.10X10^2$ log cfu/g after the treatment with kiwi 5%, kiwi 10%, lemon 0.04%, lemon 0.2 and mix (lemon 0.2% and kiwi 10%) respectively in

zero day with reduction percentages 95.64%, 89.23%, 58.97%, 99.89% and 95.51%, respectively.

By the 3rd day of the experiment, , the mean Enterobacteriacae count in control group was $1.80\times10^5 \pm 0.30X10^4$ log cfu/g and the mean count was $9.00\times10^2 \pm 0.02X10^2$, $2.5\times10^2 \pm 1.3X10^2$, $1.40\times10^3 \pm 0.60X10^3$, zero and $2.02\times10^2 \pm 0.02X10^2$ log cfu/g for the treated samples with kiwi 5%, kiwi 10%, lemon 0.04%, lemon 0.2% and mix (lemon 0.2% and kiwi 10%), respectively, with reduction percentages 98.84%, 99.67%, 98.20%, 100% and 99.7% respectively.

On the 6th day of refrigeration storage at (4±1ºC), the control samples, kiwi 5% and lemon0.04% showed extreme discoloration and off-odour indicating a complete spoilage more than other treated samples and the mean counts were $1.03\times10^2 \pm 0.02X10^2$, zero and $1.60\times10\pm 0.30X10$ log cfu/g for lemon kiwi 10%, lemon 0.2% and mix (lemon 0.2% and kiwi 10%), respectively, with reduction percentages 99.86%, 100% and 99.98 % respectively. On the 9th day of refrigeration storage at (4±1ºC), the samples of control, kiwi 5%, kiwi 10% and mix (lemon 0.2% and kiwi 10%) showed extreme discoloration and off-odour indicating a complete spoilage more than samples treated with lemon 0.2% and the mean count of them were zero log cfu/g, with reduction percentages 100%.

Table 2 Analytical results of Aerobic Plate Count (cfu\g) in chicken meat marinated with various concentrations of Kiwis and Lemon juices stored at 4±1℃

Group	Zero day	3rd	6th	9th
Control	$3.30\times10^6 \pm 3.00\times10^5$A	$5.10\times10^7 \pm 7.00\times10^6$AB	spoiled	spoiled
Kiwi (5%)	$8.60\times10^5 \pm 2.60\times10^4$C	$9.5\times10^5 \pm 3.04\times10^4$C	spoiled	spoiled
Kiwi (10%)	$3.50\times10^5 \pm 1.02\times10^4$B	$1.50\times10^5 \pm 3.00\times10^4$A	$5.00\times10^6 \pm 1.50\times10^4$AC	Spoiled
Lemon (0.04%)	$1.53\times10^5 \pm 1.00\times10^5$C	$3.12\times10^5 \pm 1.10\times10^4$C	spoiled	spoiled
Lemon(0.2%)	$5.00\times10^4 \pm 2.20\times10^3$B	$1.50\times10^4 \pm 1.00\times10^3$B	$7.00\times10^4 \pm 2.00\times10^3$B	$8.40\times10^4 \pm 3.20\times10^3$B
Lemon (0.2%)+ Kiwi (10%) mix.	$2.20\times10^5 \pm 1.00\times10^4$C	$1.20\times10^5 \pm 2.00\times10^4$C	$3.40\times10^6 \pm 2.00\times10^4$AC	spoiled

Means within a column followed by different letters are significantly different ($P < 0.05$)

Table 3 Reduction percentage 0f of Aerobic Plate Count (cfu\g) in chicken meat marinated with various concentrations of Kiwis and Lemon juices stored at 4±1℃

Group	Zero day	3rd	6th	9th
Kiwi (5%)	73.9	71.2	0	0
Kiwi (10%)	89.3	95.4	-51	0
Lemon (0.04%)	95.3	90.5	0	0
Lemon (0.2%)	98	99.5	97.8	97.4
Lemon (0.2%)+ Kiwi (10%) mix.	93.3	96.3	-3	0

Reduction count = before –after. Reduction count (%)= (before – after) ÷ before×10

Table 4 Analytical results of *Staphylococci* count (cfu\g) in chicken meat marinated with various concentrations of Kiwis and Lemon juices stored at 4±1℃ .

Group	Zero day	3rd	6th	9th
Control	$1.32\times10^4 \pm 1.3\times10^3$A	$4.0\times10^5 \pm 1.2\times10^4$D	spoiled	spoiled
Kiwi (5%)	$5.2\times10^3 \pm 0.6\times10^2$B	$2.03\times10^3 \pm 1.02\times10^2$B	spoiled	spoiled
Kiwi (10%)	$9\times10^2 \pm 4.3\times10^2$B	$3.1\times10^2 \pm 1.01\times10^2$B	$2.03\times10^2 \pm 0.1\times10^2$C	spoiled
Lemon (0.04%)	$4.6\times10^3 \pm 1.2\times10^2$B	$1.0\times10^3 \pm 0.30\times10^3$B	spoiled	spoiled
lemon (0.2%)	$4.3\times10^2 \pm 0.3\times10^2$ C	0	0	0
Lemon (0.2%)+ Kiwi (10%) mix.	$8.33\times10^2 \pm 0.06\times10^2$B	$4.03\times10^2 \pm 0.06\times10^2$B	$3.08\times10^2 \pm 2.3\times10^2$C	Spoiled

Means within a column followed by different letters are significantly different ($P < 0.05$)

Table 5 Reduction percentage of Staphylococci count (cfu\g) in chicken meat marinated with various concentrations of Kiwis and Lemon juices stored at 4±1℃.

Group	Zero day	3rd	6th	9th
Kiwi (5%)	60.60%	84.62%	0	0
Kiwi (10%)	93.18%	97.65%	98.6%	0
Lemon (0.04%)	65.15%	92.42%	0	0
lemon (0.2%)	96.74%	100%	100%	100%
Lemon (0.2%)+ Kiwi (10%) mix.	93.68%	96.94%	97.66%	0

Reduction count = before –after. Reduction count (%)= (before – after) ÷ before×10

Table 6 Analytical results of *Enterobacteriaceae* count (cfu\g) in chicken meat marinated with various concentrations of Kiwi and Lemon juices stored at 4±1℃

Group	Zero day	3rd	6th	9th
Control	$7.8\times10^4 \pm 1.06\times10^3$A	$1.8\times10^5 \pm 0.3\times10^4$A	spoiled	spoiled
Kiwi (5%)	$8.4\times10^3 \pm 2\times10^2$A	$9\times10^2 \pm 0.02\times10^2$B	spoiled	spoiled
Kiwi (10%)	$3.4\times10^3 \pm 1.3\times10^2$C	$2.5\times10^2 \pm 1.3\times10^2$B	$1.03\times10^2 \pm 0.02\times10^2$B	spoiled
Lemon (0.04%)	$3.2\times10^4 \pm 0.03\times10^3$A	$1.4\times10^3 \pm 0.6\times10^3$C	spoiled	spoiled
lemon (0.2%)	$1\times10^2 \pm 0.8\times10^2$ B	0	0	0
Lemon (0.2%)+ Kiwi (10%) mix.	$3.5\times10^3 \pm 0.1\times10^2$C	$2.02\times10^2 \pm 0.02\times10^2$ B	1.6×10±0.3×10CC	spoiled

Means within a column followed by different letters are significantly different ($P < 0.05$)

Table 7 Reduction percentage of Enterobacteriaceae count (cfu\g) in chicken meat marinated with various concentrations of Kiwis and Lemon juices stored at 4±1℃.

Group	Zero day	3rd	6th	9th
Kiwi (5%)	89.23	98.84	0	0
Kiwi (10%)	95.64	99.67	99.86	0
Lemon (0.04%)	58.97	98.20	0	0
lemon (0.2%)	99.89	100	100	100
Lemon (0.2%)+ Kiwi (10%) mix.	95.51	99.7	99.98	0

Reduction count = before –after. Reduction count (%)= (before – after) ÷ before×100

4. DISCUSSION

Microorganism contamination at various stages of the food chain is one of the major causes of food spoilage that ultimately leads to food waste, increased food insecurity and substantial economic losses. Various synthetic chemical preservatives are used to control microbial food spoilage and to extend product shelf life.

Researchers and consumers are discouraged by the use of synthetic preservatives due to their negative health impacts. Natural antimicrobials have gained attention among researchers and food manufacturers due to their safety and non-toxic status. Natural preservatives are easily obtained from plants, animals, and microbes. These naturally

occurring antimicrobial agents may be isolated from indigenous sources using a variety of advanced techniques.

4.1 Bacteriological Examination:

From the results achieved in table (2 and 3) it is evident that the initial mean count of total aerobes in untreated (control) group, was 3.30 x106 ± 7.00 x 105 such count was slightly decreased to 8.60×10^5 ± 2.60×10^4, 3.50×105±1.02×104, $1.53\times105\pm1.00\times10^5$, 5.00×10^4 ±2.20×103 and 2.20 ×105 ±1.00×104 cfu/g after the treatment with kiwi 5% ,kiwi 10%, lemon 0.04%, lemon 0.2% and mix (lemon 0.2% and kiwi 10%) respectively with reduction percentages of 73.9% ,89.3% ,95.3% ,98% and 93.3% respectively and these results are in agreement with those obtained by Yusop et al (2010), who found that the acidic marinating solutions decrease pH and inhibit microbial growth. Lemon juice was more effective in reducing total bacterial and psychrophilic bacterial counts than acetic acid and propionic acid. This might be due to the strong antioxidant activity of bioactive com-pounds in lemon juice. Lipolytic bacteria, *Salmonella Spp* and coliform bacteria were not detected in all samples and these results are constant with those recorded by Wally (2002) who found that the increase in total bacterial counts after period of storage might be due to development of more cold tolerant viable cells of some spices originally existed.

From the results achieved in table (4 and 5) it is evident that the mean count of S. aureus in untreated (control) group, was1.32X104 ± 1.30X103 log cfu/g such count was slightly decreased to 5.20X103 ± 0.60X102 , 9.00X102 ± 4.30X102 , 4.60X103 ± 1.20X102 , 4.30X102 ± 0.30X102 and 8.33X102 ± 0.06X102 log cfu/g after the treatment with kiwi 5% ,kiwi 10%, lemon 0.04% ,lemon 0.2 and mix (lemon 0.2% and kiwi 10%) respectively with reduction percentages 60.60%, 93.18%, 65.15%, 96.74% and 93.68% respectively and these results are in agreement with those obtained by Ketnawa and Rawdkuen (2011), who found that The kiwi fruit have extract which has antibacterial effect against some gram- negative and gram- positive bacteria and has effective role in meat tenderization. and have protease enzyme (actinidin) which be very effective in meat tenderization and has antifungal, antioxidant, and antibacterial effect on meat. (Koak et al., 2011) and the results came in the same line with those recorded by Ha et al., (2013) who found that kiwi fruit (Actinidia chinensis) extract has significant antibacterial activity against various Gram-positive and Gram-negative strains. Many studies have addressed the roles of these plant proteases as meat tenderizers. However, only a few of them have studied the antifungal, antioxidant, and antibacterial properties of these plant proteases on meat and poultry products.

From the results achieved in tables (6 and 7) it is evident that the mean count of Enterobacteriacae in untreated (control) group, was 7.80×104 ± 1.06X103 log cfu/g such count was slightly decreased to 3.40×103 ± 1.30X102, 8.40×103±2.00X102 , 3.20×104 ± 0.03X103, 1.00×102 ± 0.80X102 and 3.50×103 ± 0.10X102 log cfu/g after the treatment with kiwi 5%, kiwi 10%, lemon 0.04% ,lemon 0.2 and mix (lemon 0.2% and kiwi 10%) respectively with reduction percentages 95.64%, 89.23%, 58.97%, 99.89% and 95.51% respectively and these results results are in agreement with those obtained by Marwa (2014), who found that fresh breast chicken meat samples were completely free from coliform bacteria, lipolytic bacteria, *Salmonella spp* and yeast and mold, which proved the sanitary conditions of raw chicken breasts. After these periods of storage, the increase in microorganism counts might be due to the in-creasing amino acids and fatty acids produced by hydrolysis of protein and fat during storage consequently lead to suitable conditions for growth of microorganisms and the results are similar to those obtained by Frazier (1980) who found that citrus bioflavonoids also had antimicrobial properties. These compounds have reportedly wide-ranging antimicrobial properties effective against a broad range of human pathogens, fungi and food spoilage organisms.

5. CONCULOSION

The addition of lemon juice and kiwi juice improved the microbiological quality of chicken meat as they had shown antibacterial effect against Enterobacteriaceae, Staphylococcus and decrease APC counts and encourage us to conclude them to the new generation of the additives in meat industry.

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