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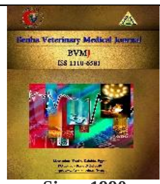

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Some organic acids as antifungal on frozen duck meat

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ABSTRACT

This study was conducted to evaluate the antifungal effect of some organic acid on frozen duck meat. The used organic acids for decontamination of frozen duck meat (breast and thigh) samples were acetic and citric acids in concentrations of (2%, 5%) and combination of both (2%) by soaking. Acetic acid (2% and 5%) decreased yeast and mold count (cfu/g) from $5.7\times10^2 \pm 0.17\times10^2$ (initial load) to $1.1\times10^2\pm 0.05\times10^2$ and $0.46\times10^2\pm 0.03\times10^2$ with a reduction percentage 80.7% and 91.9% in breast samples, and from $6.8\times10^2 \pm 0.2\times10^2$ (initial load) to $2.6\times10^2\pm 0.15\times10^2$ and $1,1\times10^2\pm 0.12\times10^2$with a reduction percentage 61.7% and 83.8% for thigh samples, respectively. Citric acid (2% and 5%) decreased yeast and mold count (cfu/g) from $5.7\times10^2 \pm 0.17\times10^2$ (initial load) to $1.4\times10^2\pm 0.18\times10^2$ and $0.56\times10^2\pm 0.03\times10^2$ with a reduction percentage 75.4% and 90.1% for breast samples, respectively, and from $6.8\times10^2 \pm 0.2\times10^2$ (initial load) to $3.4\times10^2\pm 0.14\times10^2$ and $1.3\times10^2\pm 0.08\times10^2$ with a reduction percentage 50% and 80.8% for thigh samples, respectively. While, combination of both acids (2%) decreased the count with a reduction percentage 75.4% & 90.1% in breast and 50% & 80.8% in thigh ones. Acetic acid (5%) had the highest antifungal effect, therefore, it is recommended to improve safety of duck meat.

1. INTRODUCTION

Duck meat is considered a good source of high biological value proteins for humans (Adzitey et al., 2012a) and is rich in iron, selenium, and niacin, as well as containing fewer calories than many cuts of beef (Adzitey et al., 2012b). Duck and geese production accounts for about 7.5% of the total world poultry meat production (Pigel, 2004).
Mold and yeast comprise a large group of microorganisms which are ubiquitous in nature due to easy dissemination and their vegetative spores, which are produced in large numbers and can present in the environment for a long period. Contamination of duck meat with mold starts in the environment of the slaughter halls due to a lack of hygienic measures through air, wall, floor, utensils, feather and intestinal contents of the slaughtered birds (Mansour, 1986). Contaminated feed is a main source for mold infection and mycotoxin in farm animal (Sayed et al., 2000).The incidence of meat contamination with different mold genera was investigated in different localities of the world such as Australia, Japan, Italy and Spain (Iacumin, et al., 2009; Martín-Sánchez, et al., 2011).
Fungi are not only major spoilage agents of meat results in reduction of quality with significant economic losses but also cause contamination of most food substance with secondary metabolites called mycotoxins (Adeyeye 2016). The ingestion of such mycotoxins has enormous public health significance, because these toxins are capable of causing diseases in man and animals ranging from death to chronic interference with the function of the nervous, cardiovascular, pulmonary and endocrine systems as well as alimentary tract (John and Miller, 2017)
Human exposure to aflatoxins is primarily from a consumption of contaminated food directly like cereals, seeds, fruits, etc., or indirectly by eating food products and by-products obtained from animals consuming contaminated feeds (Galvano et al., 2005).
Organic acids generally recognized as safe agents for the preservation of foods, inhibition of growth by weak acid preservatives has been proposed to be due to a number of actions including, membrane disruption, inhibition of essential metabolic reactions, stress on intracellular pH homeostasis and the accumulation of toxic anions. In yeasts, it has also been proposed that the actual inhibitory action of weak acid preservatives could be due to the induction of an energetically expensive stress response that attempts to restore homeostasis, and results in the reduction of available energy pools for growth and other essential metabolic functions (Bracey et al., 1998; Stratford and Anslow, 1998; Dalie et al., 2010).
Citric acid a white powder extracted from the juice of citrus and other acidic fruits such as lemons, limes, pineapples and gooseberries. It is also produced by the fermentation of glucose. Citric acid is highly soluble in water and primarily insoluble in fat. Citric acid was investigated for its effect on inhibition of bacteria, yeast and molds (Sorrells, 1989).
Acetic acid is commonly known as vinegar which has antimicrobial capabilities due to its ability to lower the pH

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and cause instability of bacterial cell membranes (Jay, 1992).
Therefore, the target of this work was to assess the antifungal effect of acetic and citric acids in frozen duck meat.

2. MATERIAL AND METHODS

2.1. Collection of samples:
Samples of frozen duck meat (breast and thigh (200 gm of each) were collected from different localities in El-Qalyubia governorate markets, Egypt. Samples were identified, packed and transferred to the laboratory in icebox under complete aseptic conditions without undue delay and were subjected to the mycological examination.

2.2. Organic acids used:
Acetic acid glacial 99-100% a.r. (Chem-Lab NV, Batch No. 25.5952809) that was prepared with sterile distilled water to reach (2 & 5%). Citric acid anhydrous oral (El Gomhouria Co, Batch No. 2AZ1704047) that was prepared with sterile distilled water to reach (2 & 5%).

2.3. Experimental application:
Frozen duck meat (breast and thigh) samples were thawed in chilling temperature and divided into untreated (control) and treated groups with acetic and citric acids in concentrations of (2%, 5%) and combination of both (2%) by dipping for 15 min. All groups were mycologically examined. The experiment was conducted in triplicate.

2.4. Preparation of samples (APHA, 2001)
After thawing, 25 gm of examined duck meat samples were aseptically excised and homogenized in 225 ml of sterile buffered peptone water 0.1% at 2000 rpm for 1-2 min using a sterile homogenizer. Such homogenate represents the dilution of 10^{-1}, and then decimal dilutions were done. Then the prepared samples were subjected to:
1) *Mold and yeast count (Bailey and scott, 1998)*
2) *Isolation and identification of isolated mold and yeast* based on their micromorphological properties (Pitt and Hocking 2009) .

3. RESULTS

It is evident from the result recorded in table (1) that the mean value of yeast and mold counts (cfu/g) in the examined frozen breast groups were $5.7\times10^2\pm 0.17\times10^2$, $1.1\times10^2\pm0.05\times10^2$, with a reduction percentage 80.7%, $0.46\times10^2\pm0.03\times10^2$, with a reduction percentage 91.9%, $1.4\times10^2\pm0.18\times10^2$, with a reduction percentage 75.4%, $0.56\times10^2\pm0.03\times10^2$, with a reduction percentage 90.1%, and $1.2\times10^2\pm0.11\times10^2$, with a reduction percentage 78.9% incontrol and treated with acetic acid (2%), acetic acid (5%), citric acid (2%), citric acid (5%) and a combination of both acids (2%) groups, respectively. There was a significant difference between untreated breast samples and treated ones. Moreover, the mean value of yeast and mold counts (cfu/g) in the examined frozen thigh groups were $6.8\times10^2\pm0.20\times10^2$ in control samples, while, $2.6\times10^2\pm0.15\times10^2$ with a reduction percentage 61.7% in acetic acid (2%), $1.1\times10^2\pm0.12\times10^2$ with a reduction percentage 83.8% in acetic acid (5%), $3.4\times10^2\pm0.14\times10^2$ with a reduction percentage 50% in citric acid (2%), $1.3\times10^2\pm0.08\times10^2$ with a reduction percentage 80.8% in citric acid (5%) and $1.3\times10^2\pm0.13\times10^2$ with a reduction percentage 80.8% in combination of both acids (2%). There was a significant difference between untreated thigh samples and treated ones.

Table 1 Effect of organic acids on mold and yeast count in frozen duck meat samples.

Duck samples treated with organic acids	Minimum	Maximum	Mean ± S.E.*	Red. %
Breast samples				
Control	5.4×10^2	6×10^2	$5.7\times10^2\pm0.17\times10^{2b}$	-
Acetic acid (2%)	1×10^2	1.2×10^2	$1.1\times10^2\pm0.05\times10^{2e}$	80.7
Acetic acid(5%)	4×10	5×10	$0.46\times10^2\pm0.03\times10^{2f}$	91.9
Citric acid(2%)	1.1×10^2	1.7×10^2	$1.4\times10^2\pm0.18\times10^{2e}$	75.4
Citric acid (5%)	5×10	6×10	$0.56\times10^2\pm0.03\times10^{2f}$	90.1
Acetic+ Citric(2%)	1×10^2	1.4×10^2	$1.2\times10^2\pm0.11\times10^{2e}$	78.9
Thigh samples				
Control	6.5×10^2	7.2×10^2	$6.8\times10^2\pm0.20\times10^{2a}$	-
Acetic acid (2%)	2.3×10^2	2.8×10^2	$2.6\times10^2\pm0.15\times10^{2d}$	61.7.8
Acetic acid(5%)	1×10^2	1.4×10^2	$1.1\times10^2\pm0.12\times10^{2e}$	83.8
Citric acid(2%)	3.2×10^2	3.7×10^2	$3.4\times10^2\pm0.14\times10^{2c}$	50
Citric acid (5%)	1.2×10^2	1.5×10^2	$1.3\times10^2\pm0.08\times10^{2e}$	80.8
Acetic+ Citric(2%)	1.2×10^2	1.6×10^2	$1.3\times10^2\pm0.13\times10^{2e}$	80.8

*S. E.= Standard Error of Mean. [a, b, …] values within a column with different superscript letters were significantly different at ($P \leq 0.05$). Red %= reduction percent

It is evident from the result recorded in table (2) that breast samples are free from *A. fumigatus*. while, the mean values of *A. fumigatus* (cfu/g) in the examined frozen thigh groupwere$4.6\times10\pm0.3\times10$, $2.3\times10\pm0.3\times10$, with a reduction percent 50%, $3.3\times10\pm0.3\times10$, with a reduction percent 28.2% and $3\times10\pm0.0\times10$, with a reduction percent 34.7% in control and treated with acetic acid (2%), citric acid (2%) and citric acid (5%), respectively. Furthermore, acetic acid (5%) and acetic and citric acid combination (2%) inhibited the growth of *A. fumigatus* (Red% = 100).
Also, the result in table (2) revealed that the mean values of *A. flavus* (cfu/g) in the examined frozen breast & thigh groups were $4.6\times10\pm0.6\times10$ & $6.3\times10\pm0.3\times10$ in control samples, while they were $1.3\times10\pm0.3\times10$ & $3.3\times10\pm0.3\times10$ with a reduction percent 71.7% & 47.6, in acetic acid (2%), 0 &$1.6\times10\pm0.3\times10$, with a reduction percent 100% & 74.6%, in acetic acid (5%), $2\times10\pm0.5\times10$ & $4.6\times10\pm0.3\times10$, with a reduction percent 56.5% & 26.9%, in citric acid (2%), $1\times10\pm0.0\times10$ & $3\times10\pm0.0\times10$, with a reduction percent 78.2% & 52.3% in citric acid (5%) and 0, $2.6\times10\pm0.3\times10$, with a reduction percent 100% & 58.7%, in acid combination (2%), respectively.
Moreover, the result in table (2) revealed that the mean values of *A. niger* (cfu/g) in the examined frozen breast & thigh groups were $26.3\times10\pm0.8\times10$ & $57.6\times10\pm0.3\times10$ while, they were $6.3\times10\pm0.3\times10$ & $21.6\times10\pm0.3\times10$, with a reduction percent 76 % & 62.5 %, $2\times10\pm0.5\times10$ & $2\times10\pm0.0\times10$, with a reduction percent 92.3% & 96.5%, $6.6\times10\pm0.8\times10$ & $29.6\times10\pm0.3\times10$, with a reduction percent 74.9% & 48.6%, $3.3\times10\pm0.3\times10$ & $4.6\times10\pm0.3\times10$, with a reduction percent 87.4% & 92% and $4.3\times10\pm0.3\times10$ & $8.6\times10\pm0.3\times10$, with a reduction percent 83.6 % & 85% in control and treated with acetic acid (2%), acetic acid (5%), citric acid (2%) and citric acid (5%) and a combination of both acids, respectively. Also, the result in table (2) revealed that the mean values of *Rhizopus* (cfu/g) in the examined frozen breast& thigh groups were $23.6\times10\pm0.8\times10$ & $7.6\times10\pm0.3\times10$ in control samples, while they were $4.3\times10\pm0.3\times10$ & $1.3\times10\pm0.3\times10$, with a reduction percent

81.7% & 82.8%, in acetic acid (2%), $1.6\times10\pm0.3\times10$ & 0, with a reduction percent 93.2% & 100%, in acetic acid (5%), $3.6\times10\pm0.3\times10$ & $2\times10\pm0.0\times10$, with a reduction percent 84.7% & 73.6%, in citric acid (2%), $1.6\times10\pm0.8\times10$ & 0, with a reduction percent 93.2% & 100%in citric acid (5%)and $2.6\times10\pm0.8\times10$ & $1\times10\pm0.0\times10$, with a reduction percent 88.9% & 86.8% in combination of both acids (2%) groups, respectively.

Table 2 Incidence of isolated mold genera and yeast from examined frozen breast and thigh duck meat samples treated with organic acids.

Duck samples treated with organic acids	Aspergillus flavus		Aspergillus fumigatus		Aspergillus niger		Rhizopus	
	Mean±S.E.*	Red%	Mean±S.E.*	Red%	Mean±S.E.*	Red%	Mean±S.E.*	Red. %
Breast samples								
Control	$4.6\times10\pm0.6\times10^{b}$	_	_	_	$26.3\times10\pm 0.8\times10^{c}$	_	$23.6\times10\pm0.8\times10^{a}$	_
Acetic acid (2%)	$1.3\times10\pm0.3\times10^{f}$	71.7	_	_	$6.3\times10\pm0.3\times10^{f}$	76	$4.3\times10\pm0.3\times10^{c}$	81.7
Acetic acid(5%)	_	100	_	_	$2\times10\pm0.5\times10^{h}$	92.3	$1.6\times10\pm0.3\times10^{ef}$	93.2
Citric acid(2%)	$2\times10\pm0.5\times10^{def}$	56.5	_	_	$6.6\times10\pm0.8\times10^{f}$	74.9	$3.6\times10\pm0.3\times10^{cd}$	84.7
Citric acid (5%)	$1\times10\pm0.0\times10^{f}$	78.2	_	_	$3.3\times10\pm0.3\times10^{gh}$	87.4	$1.6\times10\pm0.8\times10^{ef}$	93.2
Acetic+ Citric(2%)	_	100	_	_	$4.3\times10\pm0.3\times10^{g}$	83.6	$2.6\times10\pm0.8\times10^{de}$	88.9
Thigh samples								
Control	$6.3\times10\pm0.3\times10^{a}$	_	$4.6\times10\pm0.3\times10^{a}$	_	$57.6\times10\pm0.3\times10^{a}$	_	$7.6\times10\pm0.3\times10^{b}$	_
Acetic acid (2%)	$3.3\times10\pm0.3\times10^{c}$	47.6	$2.3\times10\pm0.3\times10^{c}$	50	$21.6\times10\pm0.3\times10^{d}$	62.5	$1.3\times10\pm0.3\times10^{f}$	82.8
Acetic acid(5%)	$1.6\times10\pm0.3\times10^{f}$	74.6	_	100	$2\times10\pm0.0\times10^{h}$	96.5	_	100
Citric acid(2%)	$4.6\times10\pm0.3\times10^{b}$	26.9	$3.3\times10\pm0.3\times10^{b}$	28.2	$29.6\times10\pm0.3\times10^{b}$	48.6	$2\times10\pm0.0\times10^{ef}$	73.6
Citric acid (5%)	$3\times10\pm0.0\times10^{cd}$	52.3	$3\times10\pm0.0\times10^{b}$	34.7	$4.6\times10\pm0.3\times10^{g}$	92	_	100
Acetic+ Citric(2%)	$2.6\times10\pm0.3\times10^{cde}$	58.7	_	100	$8.6\times10\pm0.3\times10^{e}$	85	$1\times10\pm0.0\times10^{f}$	86.8

*S.E.= Stander error of mean. Red% = reduction percentage. [a, b, …] values within a column with different superscript letters were significantly different at ($P \leq 0.05$).

4. DISCUSSION

Mold contamination of duck meat starts in the environment of the slaughter halls due to a lack of hygienic measures through air, wall, floor, utensils, feather, operators' hands and intestinal contents of the slaughtered birds (Mansour, 1986). Hence, the prevention of mold to gain access the meat is absolutely necessary. Acetic acid and citric acid are antimicrobial agents approved by USDA (FDA, 2003).

Result in table (1) revealed that yeast and mold counts of frozen duck in both breast and thigh groups were lowered by treatment with organic acids by dipping for 15 minutes in different reduction %. The effect of organic acids is mainly assigned to its ability to penetrate the cell membrane in its dissociated form, wherein more of the acid would be undissociated at lower pH than at neutral (Lin, et al. 2005) and enter the cytoplasm of the cell, dissociate within the cell and therefore decreasing the intracellular pH, providing acid-binding capacity (Roth and Kirchgessner, 1995), increasing the turgor pressure within the cell due to increase in anions from the acids and expulsion of H^{+} ions from the cell (Foster, 1999), disturbing transmembrane proton motive force, denaturating acid-sensitive proteins and DNA (Davidson, 2001) and causing an inhibition of acid sensitive enzyme (Davidson and Tylor,2007) and various essential metabolic and anabolic processes (Abee and Wouters, 1999). These actions weaken the cell. Consequently, acetic acid treated samples were the highest antifungal group than others. It interferes with cytoplasmic membrane structure and membrane proteins such that electron transport is uncoupled, and subsequent ATP production is reduced (Davidson, 2001). Moreover, as shown in tables (2) results illustrated that, acetic acid (5%) had complete and partial inhibition in growth of both of *A. flavus, A. fumigatous, A. niger & Rhizopus* in different reduction levels in both breast and thigh samples than other treatments.

Our results agree with Maehashi et al. (1996), who mentioned that acetic acid inhibits both bacteria and molds. Dorsa et al. (1997) (citric, lactic and aceti (organic acids rinses) as a single or in combination have been shown to be effective in reducing both spoilage and pathogenic microorganisms). In addition, Dubal, et al. (2003) found that spoilage organisms were highly sensitive to acid combination treatment as compared to lactic acid alone; Mohamed, et al. (2008) (reported that the four organic acids approaches (citric, lactic, acetic and tartaric) were presented as fresh beef shelf life extenders), the shelf-life of acid and acid combination treated samples was increased to 8 and 11 days as against 3 days in untreated samples. Pelaez, et al. (2012) determined that the increase of acid in the medium decreases the growth rate and extends the lag phase and Irkin, et al. (2015) who found that 2% acetic acid washing solutions showed significant inhibitory effects against yeast and molds ($P<0.05$) as they were reduced from 5.70 ± 0.08 to 4.38 ± 1.00 (log cfu/g ± standard deviation).

Regarding the effect of organic acids on Aspergillus gerera, our results in table (2) agree with Hassan et al. (2015), who found that acetic acid (10%) has the highest inhibitory effect on *A. flavus* being 45.21%, but acetic acid (5%) has (27.92%) inhibition % and citric acid (5%) gave lowest inhibition effect (0.42%). In addition, Dalie et al. (2010) who reported that, acetic acid was more effective than lactic acid and was the best inhibitor for fungi growth, El- shemy et al. (2016), who said that antimicrobial activity of acetic acid is higher than citric acid and Pundir and Jain (2010) acetic acid showed maximum antifungal activity against two isolates each of *Aspergillus. luchuensis, A. flavus, Rhizopus. stolonifer, Mucor*sp. (100%). But disagree with Sorrells (1989), who mentioned that citric acid was shown to be inhibitor than lactic acid and acetic acid.

Regarding to the effect of the used acids on *Rhizobus*, our results agree with Hassan, et al. (2015), who found that inhibition % of 5% acetic acid and 5% citric acid on Rhizopus. *Nigricans* was nearly the same (17.27% and 18.03% respectively).

5. CONCULOSION

It can be concluded that the use of acetic and citric acids potential decontaminants and acetic acid (5%) proved to be more efficient antifungal one. Therefore, recommended to improve quality and safety of duck meat.

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