

**Original Paper****Experimental Study on the Effect of *Propionibacterium* and Acetic acid on *Candida albicans* contamination in chicken fillet Stored at Chilling Conditions**Fahim, A. Shaltout¹, Ramadan, M. Salem², Eman, M. Eldiasty², Fatma, A. Diab^{3*}¹ Food Hygiene and Control Department, Faculty of Veterinary Medicine, Benha University, Egypt² Mycology Department, Animal Health Research Institute, ARC, Egypt³ Veterinarian at Benha University Hostel, Benha University, Egypt**ARTICLE INFO****Keywords**

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ABSTRACT

The current experimental study aimed to investigate the inhibitory effects of two food additives (*Propionibacterium* and acetic acid) at four different concentrations (0.5, 1.0, 2.5 and 5%) on *Candida albicans* (*C. albicans*) including recording their impact on the sensory characteristics of the treated chicken fillet samples in chilling conditions (4±1°C). After physical and microbial examination for nine days of storage, results showed significant improvement in the sensory characteristics of the treated samples, especially with increasing the concentration of the tested additives when compared with the control untreated samples, which were spoiled on the 9th day of inoculation. Regarding the anti-*C. albicans* effect of the tested materials, in general, *C. albicans* showed a higher reduction percent with increasing the concentration of the inoculated additives; furthermore, the treated samples with 2.5% and 5.0% acetic acid, after nine days of inoculation, showed more reduction in *C. albicans* counts (70.7% and 87.2%) than the treated samples with *Propionibacterium* of the same concentration (41.4% and 52.7%), respectively. Referring to the obtained results, *Propionibacterium* and acetic acid (2.5% and 5.0%) can be considered good choices for preserving and enhancing the quality of chilled chicken fillets and may be recommended for their usage in chicken fillet preservation as safe and easily applied food additives.

1. INTRODUCTION

Chicken meat is among the foods preferred by consumers in Egypt and throughout the universe because of its nutritional value and reasonable price (Abdelrahman *et al.*, 2020 and Shaltout, 2022). However, with the increased consumption of meat and meat products, the incidence of foodborne disease outbreaks linked to meat has increased significantly. Due to its qualities that can lead to quick and severe spoiling, which mostly begins at slaughterhouses through the transmission of microbes between the corpses, chicken meat is a particularly perishable commodity (Lianou *et al.*, 2017 and Shaltout, 2002).

From an economic point of view, mould and yeast are microorganisms that have a serious economic impact on the poultry meat industry throughout acceptability drawbacks because of its ability to produce extracellular proteolytic and lipolytic enzymes that initiate the protein and fat deterioration, which make it of lower nutritional value and apparently unacceptable (Mahmoud *et al.*, 2020 and Shaltout *et al.*, 2019a).

Bacteriocins or probiotics have been used in several attempts to inactivate microbial contaminants in chicken meat. Probiotics are safe food-additives defined as mono- or mixed cultures of living microorganisms that have been used in several trials to inactivate foodborne microbial contamination because of its beneficially effect in reducing

disease risk, and increasing resistance to infection through improvements in pH, color, water-holding capacity, fatty acid profile, and oxidative stability in fresh meat (Kerry *et al.*, 2018 and Saleh, 2014).

Propionibacterium is one of the probiotic's family, which is considered as an appealing candidate for advancing studies about the beneficial effect of probiotics in food industry, as it produces short-chain unsaturated fats and surface proteins through carbohydrate fermentation, that positively enhance human health (Argañaraz-Martínez *et al.*, 2013; Blasco *et al.*, 2015; Nair *et al.*, 2019 and Shaltout *et al.*, 2019b). Moreover, organic acids like acetic, citric, and lactic acids, which are recognized as safe substances for use in food production, have been frequently used to decontaminate chicken meat products due to their antimicrobial effectiveness, easily application, and it may also play an important role in the tenderness and flavoring of the processed meat (Nkosi *et al.*, 2021 and Berge *et al.*, 2001). Candidiasis represents the fourth leading cause of nosocomial infections, and mortality due to systemic candidiasis remains high, ranging from 15% to 35% depending on the infecting *Candida* species (Pal, 1997). Due to the increased number of immunocompromised people, candidiasis remains a persistent public health problem of the world. The person who are suffering from diabetes, malnutrition, cancer, HIV/AIDS, neutropenia, metabolic dysfunction, receiving antibiotics, cytotoxic

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drugs for prolonged period, and undergoing dialysis, renal transplantation, catheterization are at a greater risk of acquiring Candida infections (Pal, 2007). Candida infection can spread between patients in healthcare settings. The global incidence of Candida bloodstream infections is recorded 400,000 cases each year (Pal, 2014). The present communication delineates the growing importance of candidiasis as an opportunistic mycosis of global public health concern.

Therefore, the following study was conducted to evaluate the anti-mycotic effect of *Propionibacterium* and acetic acid of different concentrations against *C. albicans* in the experimentally infected chicken fillet, and their effects on its sensory properties.

2. MATERIAL AND METHODS

2.1. Collection of chicken fillet samples:

Raw chicken breast fillet samples (about 1,125 g) were purchased from a local poultry meat grocery in Qalubiya governorate, Egypt. The collected samples were transferred and stored aseptically in 4±1°C.

2.2. Preparation of spore suspension of *C. albicans*:

The *C. albicans* strain (Genbank accession number: AYMC2 0.00122) was used in the present study. The *Candida albicans* strain was subcultured and incubated for 48h on Malt extract agar, collected, and washed with 10 ml of sterile distilled water in 2% Tween-80. The spore suspension was standardized by plating assay (Shaltout et al., 2019a), counting and calculating to reach 10⁷ CFU/ml.

2.3. Preparation of the used additives:

Preparation of *Propionibacterium*

Propionibacterium obtained from Gencore int. inc. Ann Arbor, Mi, USA by Health Family Co., stock solution was prepared according to the product leaflet, then made other dilutions of 0.5%, 1.0%, 2.5%, and 5% by using sterile distilled water.

Acetic acid preparation

Acetic acid (99.0% conc) was obtained from Republic chemicals company, Egypt. By sterile Dist. Water, different dilutions were prepared (0.5%, 1%, 2.5% and 5% conc).

2.4. Preparation food model:

The collected fillet samples were washed and rinsed with sterile distilled water. The fresh chicken breast was cut into pieces of approximately (10 cm x 10 cm) using a sterile knife. The pieces were kept in sterile open Petri-dishes and exposed to ultraviolet rays (at 254 nm) for 15 minutes on each side to minimize the superficial commensals.

Chicken fillet samples were divided into 4 groups, the first group was considered a positive control untreated group (G1) of about 200 g weight. The 2nd (G2) and 3rd (G3) groups were each divided into four groups, about 200g weight / each (for the following treatment with the four concentrations of *Propionibacterium* and acetic acid, 0.5, 1.0, 2.5, and 5.0%). The 4th group (G4) was kept untreated in a refrigerator and used for organoleptic examination, about 500 g in weight.

2.5. Experimental procedures:

First, the G1, G2, and G3 were inoculated with *C. albicans* by dipping in the previously prepared spore suspension (10⁷ CFU/mL) for 30 minutes.

The 2nd group was subdivided into four portions. Each portion was treated with *Propionibacterium* by soaking in 2 mL of previously prepared 0.5%, 1.0%, 2.5% and 5% conc solution.

The 3rd group was subdivided into four portions. Each portion was treated with acetic acid by soaking in 2 mL of previously prepared 0.5%, 1.0%, 2.5%, and 5% conc solutions.

NB. The 1st, 2nd, and 3rd inoculated samples with *C. albicans* were incubated, before soaking in the tested additive, for 30 minutes at 25°C; then kept for another 30 minutes at room temperature (25°C) to enhance the yeast spore attachment.

All samples were stored at 4±0.2°C for 9 days and *C. albicans* counts were recorded at zero time, 48hrs, 4 days, 6 days, and 9 days.

The 4th group was kept chilled without any treatment for the organoleptic scoring.

After that, the prepared groups were subjected to the following examinations:

Organoleptic examination

Color, texture, and odor were evaluated by 3 trained panelists following the recommendations of (Collins and Huey, 2015) for color scoring, texture scoring through boiling and roasting test, and odor scoring. The color, texture, and odor of the collected samples were scored using a 9-point hedonic scale.

Determination of *C. albicans* count

After preparation of the samples following (ISO, 2017) for preparation of tenfold serial dilutions, *C. albicans* was counted according to (ISO, 2008) by pour-plate technique on duplicated Petri-dishes of malt extract agar, and then incubated in an inverted position at 37°C for 48 to 72 hrs.

Statistical analysis

After triplicate examinations of the designed treatment experiment, the obtained data were statistically evaluated by application of the Analysis of Variance (ANOVA) test according to (Feldman et al., 2003); values were presented as Mean ± standard error.

Calculation of reduction percent

$$\text{Reduction \%} = \left(\frac{B-A}{A} \right) \times 100$$

A = control reading

B = reading after treatment

3. RESULTS

According to the obtained results of sensory evaluation of the treated chicken fillet, add of *Propionibacterium* and acetic acid of different concentrations improved the physical characteristics in comparison with the control untreated samples, especially with increasing its concentration. Referring to the recorded results in Table (1), the treated groups with *Propionibacterium* and acetic acid of 2.5 and 5.0% showed high acceptability scores after the 9th day of incubation with the mild superiority of the treated samples with *Propionibacterium*, while appearing spoiled in the other tested groups.

Moreover, experimental investigation of anti-yeast effect on *C. albicans*, as recorded in Tables (2 and 3), revealed significant reduction in its count, which got higher with increasing the concentration of the tested additives along nine days of the examination. The addition of *Propionibacterium* and acetic acid (2.5 and 5.0%) showed high reduction percent with significant superiority of acetic acid (70.7 and 87.2%) than *Propionibacterium* (41.4 and 52.7%), respectively.

Referring to the obtained results, *Propionibacterium* and acetic acid showed an enhanced effect on the sensory and microbial count appeared as elongated shelf-life with lower

microbial count in comparison with the control untreated group.

Table 1 Sensory evaluation of the treated groups compared with control group.

Groups	Parameter	Zero time	2 nd day	4 th day	6 th day	9 th day	
Control	Color	++++	+++	++	+	S.	
	Odor	++++	+++	++	+	S.	
	Texture	++++	++++	+++	+	S.	
Propionibacterium	0.5%	Color	++++	+++	++	+	S.
		Odor	++++	+++	++	+	S.
		Texture	++++	++++	+++	+	S.
	1.0%	Color	++++	+++	++	+	S.
		Odor	++++	+++	++	+	S.
		Texture	++++	++++	+++	+	S.
	2.5%	Color	++++	++++	++++	+++	+++
		Odor	++++	++++	++++	+++	+++
		Texture	++++	++++	++++	+++	+++
5.0%	Color	++++	++++	++++	++++	++++	
	Odor	++++	++++	++++	++++	++++	
	Texture	++++	++++	++++	++++	++++	
Acetic acid	0.5%	Color	++++	+++	++	+	S.
		Odor	++++	+++	++	+	S.
		Texture	++++	+++	++	+	S.
	1.0%	Color	++++	++++	+++	++	S.
		Odor	++++	++++	+++	++	S.
		Texture	++++	++++	+++	++	S.
	2.5%	Color	++++	++++	++++	+++	+++
		Odor	++++	++++	++++	+++	+++
		Texture	++++	++++	++++	+++	+++
5.0%	Color	++++	++++	++++	+++	+++	
	Odor	++++	++++	++++	+++	+++	
	Texture	++++	++++	++++	+++	+++	

++++: excellent +++: very good ++: good +: bad S.: spoiled

Table 2 Antifungal activity of various concentrations of Propionibacterium and Acetic acid different treated fillet chicken meat during storage at 4±10C.

Treat	Control	P 0.5%	P 1.0%	P 2.5%	P 5.0%	A 0.5%	A 1.0%	A 2.5%	A 5.0%
Zero	6.49±0.01 ^a								
2 nd day	8.1±0.1 ^a	5.9±0.1 ^b	5.5±0.1 ^{bc}	5.3±0.1 ^{cd}	5.1±0.1 ^e	5.0±0.04 ^e	3.6±0.3 ^f	2.4±0.04 ^g	1.2±0.1 ^h
4 th day	6.5±0.2 ^a	5.4±0.1 ^b	5.1±0.06 ^{bc}	4.7±0.1 ^c	3.7±0.1 ^d	4.0±0.01 ^d	3.1±0.1 ^e	2.3±0.1 ^f	1.03±0.05 ^g
6 th day	5.9±0.1 ^a	5.5±0.2 ^b	4.9±0.03 ^{bc}	4.5±0.04 ^{cd}	3.1±0.08 ^d	S.	3.8±0.03 ^d	2.0±0.09 ^e	0.79±0.01 ^f
9 th day	S	S	S	3.8±0.03 ^a	3.07±0.2 ^b	S	S	1.9±0.08 ^c	0.83±0.07 ^d

P. Propionibacterium; A. Acetic acid; S. Spoiled

Results are expressed as mean ± S.E.M.

a, b & c: There is no significant difference (P>0.05) between any two means, within the same row (of each group) have the same superscript letter.

Control: Untreated group.

P 0.5%: Treated group with *Propionibacterium* (0.5%) P 1.0%: Treated group with *Propionibacterium* (1.0%) P 2.5%: Treated group with *Propionibacterium* (2.5%)

P 5.0%: Treated group with *Propionibacterium* (5.0%) A 0.5%: Treated group with Acetic Acid (0.5%) A 1.0%: Treated group with Acetic Acid (1.0%)

A 2.5%: Treated group with Acetic Acid (2.5%) A 5.0%: Treated group with Acetic Acid (5.0%)

Table 3 Reduction % of total yeast (C. albicans) count in treating fillet chicken meat.

Treat	P 0.5%	P 1.0%	P 2.5%	P 5.0%	A 0.5%	A 1.0%	A 2.5%	A 5.0%
Zero	--	--	--	--	--	--	--	--
48h (2 nd day)	9.1	15.3	18.6	21.4	23.0	44.5	63.0	81.5
96h (4 th day)	16.8	21.4	27.6	43.0	38.4	52.2	64.6	84.5
144h (6 th day)	15.3	24.5	30.7	52.2	--	41.4	69.2	87.8
216h (9 th day)	S.	S.	41.4	52.7	S.	S.	70.7	87.2

P. Propionibacterium; A. Acetic acid; S. Spoiled

Reduction percent = $\left(\frac{B-A}{A}\right) \times 100A$ = control reading from Table (2) B = reading after treatment from Table (2)

P 0.5%: Treated group with *Propionibacterium* (0.5%) P 1.0%: Treated group with *Propionibacterium* (1.0%) P 2.5%: Treated group with *Propionibacterium* (2.5%)

P 5.0%: Treated group with *Propionibacterium* (5.0%) A 0.5%: Treated group with Acetic Acid (0.5%) A 1.0%: Treated group with Acetic Acid (1.0%)

A 2.5%: Treated group with Acetic Acid (2.5%) A 5.0%: Treated group with Acetic Acid (5.0%)

4. DISCUSSION

The introduction of new additives and/or techniques in the processed meat industry to improve the nutritional and shelf-life quality of the meat products while keeping the consumer acceptability is a new challenge nowadays (Shaltout *et al.*, 2019a and Ursachi *et al.*, 2020).

Large amounts of food and feed are lost yearly because of mould and yeast spoilage. Bio-preservation by *Propionibacterium* has gained increased interest and might be particularly useful due to its important role in many food fermentations. *Propionibacterium* plays an antifungal effect in the food industry, which can be attributed to the organic acids produced by these bacteria (Shaltout and Edris, 1999). (Lind *et al.*, 2005) tested the antifungal activities of various *Propionibacterium* strains against eight food- and foodborne mould and yeasts and found a significant reduction in the tested mould and yeast strains, especially with lower pH values due to the secreted propionic acid, with acetic acid being the most potent antifungal acid.

Propionibacterium spp., a cutting-edge probiotic, may be credited with improving the sensory qualities of the treated groups because they can use lactose and lactates as carbon sources, secrete intracellular peptidases and cell wall associated proteases, synthesis compounds with preservatives properties (bacteriocins, propionic acid, and acetic acid), and they produce compounds with aroma and flavor. Furthermore, the recorded reduction in *C. albicans* can be referred to its ability to secrete bacteriocins, propanoic acid and vitamin B12 that have direct antifungal effects (Shaltout *et al.*, 2019b; Shaltout *et al.*, 2019a and Turgay *et al.*, 2020).

As acidifier, color diluent, curing and pickling agent, pH control agent, solvent, and preservative, acetic acid has been used in foods as a flavor enhancer and flavoring agent. It is generally recognized as safe when used in accordance with good manufacturing practice (FDA, 2012). The obtained results came in agreement with those recorded by Northcutt *et al.* (2000), Serdaroğlu *et al.* (2007) and Shewail *et al.* (2018), who showed improvement in the sensory parameters of meat after the addition of acetic acid; while disagreeing with the results of Nadzirah *et al.* (2016) and Smith and Young (2007), who reported some changes in the color of the treated chicken meat.

Acetic acid is typically utilized as secure food preservative; they lower the cytoplasmic pH and halt metabolic activities. However, organic acids operate on the plasmic membrane to kill sensitive organisms by neutralizing its electrical potential and increasing its permeability (Dalie *et al.*, 2010 and Shaltout *et al.*, 2016). Some methods explain how organic acids' inhibitory mode causes pH to decrease, which may affect the development by acidifying the cell and requiring a lot of energy to maintain intracellular pH equilibrium (Pandey *et al.*, 2016). Other possibilities have also been put up, such as membrane disruption, metabolic processes being stopped, and the buildup of poisonous anions (Lind *et al.*, 2005). This hydrophobic property of the majority of organic acids, which permit unhindered transport of the protonized form across the cell membrane, was thought to be responsible for this phenomenon. The gradients in pH and osmolarity between the inner and outer surfaces of the cell cause this diffusion process to take place. The acid undergoes dissociation as soon as it enters the cytoplasm, which lowers the intracellular pH by

releasing protons. The intracellular pH is greater than the external pH. The cell devotes most of its energy content to eliminate these newly produced protons to overcome the drop in cytoplasmic pH brought on by the ionization of the ingested acid, which causes slower growth kinetics (Pelaez *et al.*, 2012).

The obtained inhibitory effects of *Propionibacterium* and acetic acid on *C. albicans* came in agreement with El-Shafei *et al.* (2008), who reported that the potential of the tested *Propionibacterium* protective cultures to inhibit yeast growth on Kareish cheese (soft cheese) was a promising finding to be used in further processed food industries. While, Hassan *et al.* (2015) who examined the antifungal effects of many organic acids at different fungal growth and with variable concentration and detected that acetic acid (10%) has the highest inhibitory effect on the examined strains (45.21%) where the final pH was 3.25; Osman (2016) who recorded a significant improvement in the sensory quality with a significant reduction in yeast counts after 21 days of cold storage in chicken fillet after acetic acid treatment; Saleh *et al.* (2021) who recorded a significant reduction in the yeast count after treating with acetic acid in fresh meat. In addition, Pelaez *et al.* (2012) determined that the increase of acid in the medium decreases the growth rate and extends the lag phase of the tested microorganisms.

5. CONCLUSION

It can be suggested that the use of *Propionibacterium* and acetic acid as preservatives for the chicken fillet, helps in increasing its shelf life over a wide range of time.

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