Experimental Study on the Effect of Propionibacterium and Acetic acid on Candida albicans contamination in chicken fillet stored at Chilling Conditions

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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 ($4 \pm 1^{O}C$). 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 than the treated samples with *Propionibacterium* of the same concentration. Referring to the obtained results, *Propionibacterium* and acetic acid (2.5% and 5.0%) could 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 [1, 2]. However, with increased consumption of meat and meat products, the incidence of foodborne disease outbreaks linked to meat has increased significantly [3, 4]. Due to its qualities that can lead to quick and severe spoiling, which mostly begins at slaughterhouses through transmission of microbes between the corpses, chicken meat is a particularly perishable commodity [5–7].

From the economic point of view, mould and yeast are one of microorganisms that have serious economic impacts on the poultry meat industry throughout its drawbacks in acceptability and health concerns. Mould and yeast commonly produce extracellular proteases and lipases that can initiate and catalyze the deterioration and breakdown of bonds in proteins and lipids into their original amino acids and fatty acids [8, 9].

Bacteriocins or probiotics have been used in several attempts to inactivate microbial contaminants in chicken meat [10]. Probiotics are new green food-additives defined as mono-or mixed cultures of living microorganisms that beneficially help 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 [11, 12].

The *Propionibacterium* family, which includes *P. freudenreichii* and *Acidipropionibacterium thoenii*, is an appealing candidate for the advancement of probiotic studies because it produces short-chain

unsaturated fats via carbohydrate fermentation [2, 13, 14], and surface proteins that positively enhance human health [7, 15].

Additionally, due to the effectiveness of their antimicrobial properties, and ease of application, organic acids (OA) like acetic, citric, and lactic acid - which recognized as safe substances (GRAS) for use in food production are frequently used to decontaminate chicken meat products [16]. It may also play an important role in the tenderness and flavor of processed meat[17].

Therefore, the aim of the following study was to illustrate the anti-mycotic effect of probiotic (*Propionibacterium*) and acetic acid at different concentrations in the chicken fillet and their effects on the sensory characteristics of meat.

2. Materials And Methods

2.1. Collection of chicken fillet samples

Raw chicken breast fillet samples were purchased from a local poultry meat grocery in Giza city, Egypt. The collected samples were transferred and stored aseptically in 4 ± 1^{O}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 [9], counting and calculating to reach to 10^7 CFU/ml.

2.3. Preparation of the used additives

2.3.1. Preparation of *Propionibacterium*

Propionibacterium obtained from Gencore int. inc. Ann Arbor, Mi, USA by Health Family Co., stoke solution was performed according to the product leaflet, then made another dilution of 0.5%, 1.0%, 2.5%, and 5% by using sterile dis. water.

2.3.2. 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 considered as 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 of all, the G1, G2 and G3 were inoculated with *C. albicans* by dipping in the previously prepared spore suspension (10^7 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^OC; then kept for another 30 minutes at room temperature (25^OC) to enhance the yeast spore attachment.
All samples were stored at 4 ± 0.2^OC 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:

I. Organoleptic examination

Color, texture, and odor were evaluated by 3 trained panelists following the recommendations of **Collins and Huey** [18] 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**.**

II. Determination of *C. albicans* count

After preparation of the samples following **ISO 6887-2** [19] for preparation of tenfold serial dilutions, *C. albicans* was counted according to **ISO 21527-1** [20] by pour-plate technique on duplicated petri dishes of Malt extract agar, and then incubated in an inverted position at 37^OC for 48 to 72 hrs.

III. Statistical analysis

After triplicate examination of the designed treatment experiment, the obtained data were statistically evaluated by application of Analysis of Variance (ANOVA) test according to **Feldman *et al.*** [21]**;** values were presented as Mean ± standard error.

3. Results

According to the obtained results of sensory evaluation of the treated chicken fillet, addition 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)**, treated groups with *Propionibacterium* and acetic acid of 2.5 and 5.0% showed high acceptability score after the 9th day of incubation with mild superiority of the treated samples with *Propionibacterium*, while appeared spoiled in the other tested groups.

Table (1): Sensory evaluation of the treated groups comparing with control group.

Groups		Parameter	Zero time	2nd day	4th day	6th day	9th day
Control		Color	++++	+++	++	+	S.
		Odor	++++	+++	++	+	S.
		Texture	++++	++++	+++	+	S.
Propionobacterium	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

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. 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.

Table (2): Antifungal activity of various concentration of different treated fillet chicken meat during storage at 4 ± 1^{O}C

Treat / Time	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}	6.49±0.01^{a}	6.49±0.01^{a}	6.49±0.01^{a}	6.49±0.01^{a}	6.49±0.01^{a}	6.49±0.01^{a}	6.49±0.01^{a}	6.49±0.01^{a}
2nd 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}
4th 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}
6th 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}	Spoiled.	3.8±0.03^{d}	2.0±0.09^{e}	0.79±0.01^{f}
9th day	Spoiled	Spoiled	Spoiled	3.8±0.03^{a}	3.07±0.2^{b}	Spoiled	Spoiled	1.9±0.08^{c}	0.83±0.07^{d}

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

Treat / Time	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 (2nd day)	9.1	15.3	18.6	21.4	23.0	44.5	63.0	81.5
96h (4th day)	16.8	21.4	27.6	43.0	38.4	52.2	64.6	84.5
144h (6th day)	15.3	24.5	30.7	52.2	--	41.4	69.2	87.8
216h (9th day)	S.	S.	41.4	52.7	S.	S.	70.7	87.2

4. Discussion

The introduction of new additives and/or techniques to the processed meat industry in order to improve the nutritional and shelf-life quality of the meat products while keeping the consumer's acceptability is a new challenge nowadays [9, 22].

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 the food industry, which can be attributed to the organic acids produced by these bacteria [23]. **Lind** et al. [24] tested the antifungal activities of various *Propionibacterium* strains against eight food- and feedborne 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, secret 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 [7, 9, 25].

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[26].

The obtained results came in agreement with those recorded by **Northcutt** et al. [27]; **Serdaroğlu** et al. [28] **and Shewail** et al. [29], who showed improvement in the sensory parameters of meat after the addition of acetic acid; while disagreed with the results of **Nadzirah** et al. [30] and **Smith and Young 31]**, who reported some changes in the color of the treated chicken meat.

Acetic acid is typically utilized as secure food preservative; they lower 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 [32, 33]. Some methods explain how organic acids' inhibitory mode causes pH to decrease, which may affect development by acidifying the cell and requiring a lot of energy to maintain intracellular pH equilibrium [34]. Other possibilities have also been put up, such as membrane disruption, metabolic processes being stopped, and the buildup of poisonous anions [24]. 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 the majority of its energy content to eliminate these newly produced protons in order to overcome the drop in cytoplasmic pH brought on by the ionization of the ingested acid, which causes slower growth kinetics [35].

The obtained inhibitory effects of Propionibacterium and acetic acid on *C. albicans* came in agreement with **El-Shafei** et al. [36], 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. In this research; **Hassan** et al. [37] 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** [38] 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. [39] who recorded a significant reduction in the yeast count after treating with acetic acid in fresh

meat. In addition, **Pelaez** et al. [35] determined that the increase of acid in the medium decreases the growth rate and extends the lag phase of the tested microorganisms.

Therefore, 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.

Declarations

Author's contribution List

FAD collected samples and food grade additives, performed the practical part of the research in guidance of RMS and EME, performed the required statistical tests and typed them in tables, typed the manuscript in this form, and uploaded and followed-up the research publication. FAS, RMS and EME developed the research plan, and supervised its implementation. All the entire authors reviewed the research before publication.

Data availability

All data available are available from the corresponding author upon requist.

Conflict of Interest

The authors state that the publishing of this paper does not include any conflicts of interest.

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