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Research Article

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Experimental Studying the Effect of *Propionibacterium* and Acetic Acid on *Candida Albicans* Contaminating Chicken Fillet in Chilling Conditions

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Abstract

Raw meat spoilage by yeasts is a significant problem that is a consequence of many yeast species growth in the product including Candida albicans. Candida albicans utilizes food components and are transformed into many metabolic end products leading to sensory, chemical and physical properties changes with especial reference to the health impacts on the consumer's health. Therefore, the current experimental study aimed to investigate the inhibitory effect of two vital food additives (Propionibacterium and acetic acid) at four different concentrations (0.5, 1.0, 2.5 and 5%) on C. albicans including recording their impact on the sensory characters of the treated chicken fillet samples in chilling conditions (4±1^{O}C): After physical and microbial examination for nine days of storage, results showed significant improvement in the sensory characters of the treated samples, especially with increasing the concentration of the tested additives when compared with the control untreated samples which was spoiled at the 9th day of inoculation. Regarding with the anti-C. albicans effect of the tested materials, in general, C. albicans showed 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%, respectively) 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 of the 2.5% and 5.0% concentration could be considered a good choice for preservation and enhancing quality of chilled chicken fillet, and may be recommended for its usage in chicken fillet preservation as safe and easy applied food additives.

Keywords: Probiotic, Organic Acid, Meat Products, *Candida Albicans*

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]. But, with increasing consumption of meat and meat products, the number of foodborne pathogen outbreaks related to meat has significantly increased [3, 4].

Particularly, chicken meat is a highly perishable product because of its characteristics that can cause rapid and intensive spoilage, which mainly starts at slaughterhouses through spread of microorganisms between carcasses [5-8].

From the economic point of view, mould and yeast are one of microorganisms that have serious economic impacts in poultry meat industry throughout its drawbacks on the 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 protein and lipids into their original amino acids and fatty acids [9, 10].

Numerous efforts have been made to inactivate microbial contaminants in chicken meat using bacteriocins or probiotics [11]. Probiotics are new green food-additives defined as mono-or mixed cultures of living microorganisms that beneficially help in reduce disease risk, and increasing resistance to infection through improvement in pH, colour, water-holding capacity, fatty acid profile and oxidative stability in fresh meat [12, 13].

The family *Propionibacterium,* including *P. freudenreichii, Acidi Propionibacterium thoenii, A. jensenii, and A. acidipropionici*, is an appealing contender for the improvement of probiotic studies as it produces short chain unsaturated fats by the meaning of carbo-

hydrate fermentation, and surface proteins that positively enhance human health [7, 14-18].

In addition, organic acids (OA) such as acetic, citric, and lactic acid, which recognized as safe substances (GRAS) for in food production are commonly used for decontamination of chicken meat products due to their antimicrobial potency, cost-effectiveness, and application simplicity [19]. It may also play an important role in tenderness and flavor of processed meat [20].

Therefore, the aim of the following study is to illustrate the anti-mycotic effect of probiotic *(Propionibacterium)* and Acetic acid added by different concentrations in the chicken fillet and their effect in the sensory characters of meat.

Materials and Methods

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.

Preparation of Spore Suspension of Yeast Culture *(C. albicans)*

The *Candida albicans* strain (Genbank accession number: AYMC2 0.00122) was used in the present study. The used strain was previously isolated at Mycology Department, Animal health Research institute, ARC, Egypt. *Candida albicans* strain was subcultured and incubated for 48 h on Malt extract agar, and then it was collected and washed with 10 ml of sterile distilled water in 2% Tween-80 with the aid of glass beads to help in the spore dispersion. The spore suspension was standardized by plating assay, counting and calculating to reach to 10^7 CFU/ml [18].

Preparation of the Used Additives

Preparation of *Propionibacterium*

Preparation of *Propionibacterium*, obtained from Gencore int. inc. Ann Arbor, Mi, USA by Health family company, 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 distal 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.).

Preparation Food Model

The collected fillets samples were washed and rinsed with sterile distilled water. The fresh chicken breast cut into pieces of approximately (10 cm x 10 cm size) using a sterile knife. The pieces were kept in sterile open petri dishes and exposed to ultra violet rays (at 254 nm) for 15 minutes each side for maximizing reduction of the superficial commensals.

Chicken fillet samples were divided into 4 groups, where
First group considered as positive control untreated group (G1) about 200 g weight.
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% conc."):
While, 4th group (G4) was kept untreated in a refrigerator and used for organoleptic examination, about 500 g weight.

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.
• 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.
• 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. Solution.
NB. The 1st, 2nd and 3rd inoculated samples with *C. albicans* were incubated, before soaking in the tested additive, for 30 minutes at 25^{O}C; then kept for another 30 minutes at room temperature (25^{O}C) to enhance the yeast spore attachment.
All samples were stored at 4±0.2^{O}C for 9 days and *C. albicans* counts were recorded at zero time, 48h, 4days, 6 days and 9 days.
• The 4thgroup 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 Gracey and Collins (1992) and Hunt et al. (1991) for color scoring; Gracey (1986) and Miller (1994) for texture scoring through boiling and roasting test; and Gracey (1986) and Miller (1994) for odor scoring. The color, texture and odor of the collected samples were scored following 9-point hedonic scale (Anna, 1993).

Determination of *C. albicans* Count was Performed According to ISO 21527-1 (2008)

Twenty-five grams from each sample were carefully and aseptically homogenized in a blinder after mixing with 225 ml of sterile peptone water 0.1% to form a dilution of 1:10 from which tenfold dilution were accomplished. Accurately, one ml from each previously prepared serial dilution were separately poured into duplicated petri dishes carefully and mixed with 15 ml of Malt extract agar which tempered at 45±1^{O}C for estimation of *C. albicans* count. After solidification the inoculated plates were incubated in an inverted position at 37^{O}C for 48 to 72 hrs.

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, values were presented as Mean ± standard error.

Results

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

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

Discussion

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 with keeping the consumer's acceptability is a new challenge nowadays [9, 21].

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 their important role in many food fermentations. *Propionibacterium* plays an antifungal effect in food industry which can be attributed to the produced organic acids by these bacteria [22]. Lind et al. evaluated the antifungal activity of different Propionibacterium strains against eight food- and feed borne mould and yeasts; and recorded a significant reduction in the tested mould and yeast strains, especially with lower pH values resulted from the secreted propionic acid, followed by acetic acid, was the most potent antifungal acid [23].

In the present study, *Propionibacterium* and acetic acid of four different concentrations (0.5%, 1.0%, 2.5% and 5.0%) were evaluated for keeping quality of raw chicken fillet represented by organoleptic examination of the treated samples in comparison with control untreated samples; in addition, anti-yeast investigation was studied on experimentally inoculated samples with *C. albicans* in chilling conditions (4±0.2^{o}C) for 9 days.

According to the obtained results of sensory evaluation of the treated chicken fillet samples after nine days of cold storage, addition of *Propionibacterium* and acetic acid of different concentrations showed an improvement in the physical characters of the treated samples 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.

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.

Improvement of the sensory characteristics of the treated groups with *Propionibacterium* spp., among innovative probiotics, may be referred to its valuable benefits from the technological point of view, as they can utilize lactose and lactates as carbon source, secrete intracellular peptidases and cell wall associated proteases, synthesize compounds that have preservative properties (bacteriocins, propanoic acid, and acetic acid), they produce compounds that have aroma and taste; they also have the capacity to convert free amino acids to aromatic compounds), and are capable of production of vitamin B12.Furthermore, the recorded reduction in *C. albicans* can be referred to its ability to secret bacteriocins, propanoic acid and vitamin B12 that have direct antifungal effect [7, 18, 24, 25].

Acetic acid has been used in foods as a flavor enhancer and flavoring agent; an acidifier, color diluent, curing, and pickling agent, pH control agent, solvent, and preservative. It is generally recognized as safe when used in accordance with good manufacturing practice [26]. The obtained results came in agree with those recorded by Northcutt et al.; Serdaroğlu et al. and Shewail et al., who showed improvement in the sensory parameters of meat after addition of acetic acid [27-29]. While disagreed with the results of Nadzirah et al. and Smith and Young who reported some changes in color of the treated chicken meat [30, 31].

Acetic acids generally used as safe agents to preserve foods, these acids reduce cytoplasmic pH and stop metabolic activities. On the other hand, organic acids cause the death by the susceptible organisms act on the plasmic membrane by neutralizing its electrochemical potential and increasing its permeability [32, 33]. Some mechanisms explained the inhibitory mode of organic acids resulting in pH decreasing, this may influence the growth by acidifying the cell, which will consume a great amount of energy to maintain the intracellular pH homeostasis [34]. Other explanations have also been proposed including the membrane disruption, the interruption of metabolic reactions, and the accumulation of toxic anions [23]. This phenomenon was attributed to the hydrophobic feature of most organic acids, which allows free diffusion of the protonized form through cell membrane. This diffusion process occurs directly due to pH and osmolarity gradients that exist between the

inner and outer sides of the cell. The intracellular pH is higher than the extracellular, and the acid undergoes dissociation as soon as it enters the cytoplasm and then decreases the intracellular pH by releasing the proton. In order to counter the decrease of cytoplasmic pH, resulting from the ionization of the entered acid, the cell allocates the main part of its energy content to eliminate these newly formed protons which results in slower growth kinetics [35].

The obtained inhibitory effects of Propionibacterium and acetic acid on *C. albicans* came in agree with El-Shafei et al. who recorded 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 [36]. Hassan et al. who examined the antifungal effect 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 [37]. Osman who recorded a significant improvement in the sensory quality with reduction in yeast counts along 21 days of cold storage in chicken fillet after acetic acid treatment [38]. Saleh et al. who recorded a significant reduction in the yeast count after treating with acetic acid in fresh meat [39]. In addition, Pelaez et al. determined that the increase of acid in the medium decreases the growth rate and extends the lag phase of the tested microorganisms [35].

Therefore, it can be suggested that the use of *Propionibacterium* and acetic acid as preservative for the chicken fillet help in increase its shelf life over a wide range of time.

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