Received: 01 October 2023, Accepted: 23 October 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 10, 1960-1965

Probiotics Blueprint for Meliorating the Quality Aspects of Chicken Nuggets

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Abstract

This study aimed to evaluate the effect of probiotics on the microbial and physiochemical quality of chicken nuggets. In the current investigation, different concentrations of *Bacillus clausii* and *Saccharomyces cerevisiae* were added as probiotics to chicken nuggets. The proximate composition, physicochemical properties, and microbial quality of prepared chicken nuggets were assessed over 21 days at -18°C. The findings of this investigation demonstrated the ability of probiotics such as *Bacillus clausii* and *Saccharomyces cerevisiae* to depress the growth and multiplication of aerobic and psychotrophic bacteria as well as the yeast and mold count over the storage period. Varying preparations influenced the mean pH, peroxide, and TBA values during the storage of chicken nuggets ($p \leq 0.05$). There was a significant decrease in moisture, protein, fat, and ash content compared to the control. In conclusion, additive supplementation of *Bacillus clausii* and *Saccharomyces cerevisiae* improved the chicken nuggets' shelf life attributes, physicochemical characteristics, and microbial quality.

KEYWORDS
Bacillus probiotic, *Saccharomyces cerevisiae*, Chicken nuggets, Quality, Probiotic

INTRODUCTION

Chicken products play a significant role in the human diet due to their beneficial nutritious properties such as low lipid content, high biological value proteins, essential amino acids, and a natural source of vitamins B2, B3, and B6 as well as minerals such as Fe, P, K, Zn, and Se (BEDCA/AESAN, 2019). Currently, a large proportion of chicken meat and chicken products are expended in the form of "fast food" or "ready-to-eat" products, such as chicken nuggets, due to their numerous advantages, such as reduced preparation period, low price, and long shelf life when frozen (Abd-El-Aziz *et al*., 2021). As a result, there is a growing demand for chicken nuggets concerning quality, flavor, and nutritional value. This has resulted in using binders and extenders to satisfy consumer demand and reduce production costs (Opeyemi *et al*., 2022). Creating and consuming probiotic food products is a growing worldwide customer trend that has significantly impacted the functional food market. Nonetheless, functional foods such as food products holding probiotic microbes represent an exceptional opportunity and a promising market for the meat industry to enhance the quality of meat and meat products and develop healthier alternatives (Jafari *et al*., 2017; Saleh *et al*., 2017; Sallam *et al*., 2020; Elabbasy *et al*., 2021).

Due to immune stimulation, antimicrobial activities, and competitive exclusion, the use of *Bacillus* species spores as probiotic dietary supplements is rapidly growing (Katsutoshi *et al*., 2011; Morshdy *et al*., 2022). Related to other probiotic bacterial strains, probiotic *Bacillus* strains have better viability, survivability, and probiotic properties during demanding manufacturing procedures (like cooking), the product's storage, and passage through the gastrointestinal tract (Gallego and Salminen, 2016). Therefore, processing techniques, raw materials, and ingredient elements have a substantial impact on a nugget's overall quality, making consumers prefer nuggets with a high nutritive value, low cholesterol, good textural properties, and a pleasant flavor and taste profile (Opeyemi *et al*., 2022). As a result, this study aimed to investigate the influence of *Bacillus clausii* and *Saccharomyces cerevisiae* on the overall quality of chicken nuggets.

MATERIALS AND METHODS

Ethical approval

Ethical approval is not required for this study.

Samples

Fresh, boneless, skinless, 150 chicken breast fillets (200 g each) were obtained from poultry markets, Faiyoum Governorate, Egypt, during February and March 2022. Plain flour, refined salt, Egg, white pepper powder, fresh garlic paste, fresh onion, and commercial Baker's yeast (*Saccharomyces cerevisiae*) were purchased from the local market in Faiyoum Governorate, Egypt. Dried breadcrumbs (carbohydrates 81.36%, protein 12.32%, fat 3.73%, moisture 6.5%, ash 0.93%, and fiber 0.79) were obtained from the Modern Bakeries (Rich Bake) company, Faiyoum, Egypt. Spores of poly-antibiotic resistance *Bacillus clausii* (2 milliards/5ml) from Sanofi Co.

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Table 1. Nugget ingredients.

Ingredient	Quantity used
Chicken boneless	500 g
Egg	1
Black pepper	12 g
Garlic paste	15 g
Onion	150 g
Plain flour	120 g
Breadcrumbs	70 g
Salt	15 g

Preparation of chicken nuggets

One hundred fifty chicken nugget pieces were prepared at the laboratory of the Department of Food Hygiene of the Faculty of Veterinary Medicine, University of Aswan as described by Faiz *et al*. (2020) and divided into five groups; each group included 10 samples with three replications of each treatment. The nugget components were weighed and combined following the recipe (Table 1). The beef was cleaned under running water, handdeboned, and then minced in an electric mincer (MG 100). All other ingredients were added as directed and blended in a meat mixer to create a consistent mixture after the broiler meat and onions had been combined for five minutes. Five different formulations of the chicken nuggets were prepared as follows: a control group (T1) without any treatment, a group treated with spores of poly-antibiotic resistance *Bacillus clausii* (2 milliards/5ml, Sanofi co.) with two different concentrations of 1% (T2) and 3% (T3) and a group treated with *Saccharomyces cerevisiae* (Commercial Baker's yeast) with two different concentrations of 1% (T4) and 3% (T5). To form a nugget, the mixture was stretched out into a thin sheet (10 mm thickness) and formed into discs measuring 3 cm in diameter and weighing 50 g each. The nuggets were coated separately in plain flour and breadcrumbs and then baked at 180°C until they reached the desired color and texture. All samples were packaged into FoodSaver vacuum seal bags (Vac 1075 by FoodSaver). The nuggets were kept at -18°C for 21 days to conduct additional analyses for various parameters at 0, 7, 15 and 21 days after storage. The chicken nugget items' manufacturing, packing, and storage all followed hygienic procedures.

Microbial quality

The American Public Health Association's (APHA, 2001) methodologies determined all the microbiological parameters. To make a serial dilution of samples, about 10 g of material was aseptically weighed and homogenized for 2 min., in a sterile mortar with 90 ml of 0.1% sterile peptone water. Using plate count agar (HiMedia, M091) incubated at 37±1°C for 48 h for total plate count and at 4±1°C for psychrophilic count, whereas potato dextrose agar (HiMedia, M096) incubated at 25ºC for 5-7 days for yeast and mold count. Triplicates of all analyses were performed on days 0, 7, 15, and 21.

Physiochemical quality

pH

Five g of the prepared chicken nuggets were homogenized in a blender with 20 mL of distilled water for 1 minute. All measurements were carried out in triplicate, and the results were presented as the mean and standard deviation (Kim *et al*., 2015).

Peroxide value (PV)

According to AOCS (1997), 3 g was heated in a water bath (60°C for 3 min) before being vigorously agitated for 3 min with 30 ml of acetic acid–chloroform solution (3:2, v/v) and potassium iodide solution (1 ml). The reaction mixture was titrated with a standard sodium thiosulfate solution (25 g/L) after standing in the dark for 5 min. The following equation was used to compute the PV as meq/kg sample: PV (meq/kg) = (S×N)/kg× 100 where S is the volume of titration (ml), N is the normality of sodium thiosulfate solution (N = 0.01), and W is the sample weight (kg).

Thiobarbituric acid (TBA) value

Thiobarbituric acid (TBA) was dissolved in a stock solution of 0.37% TBA, 15% TCA, and 0.25 N HCl by slowly heating the mixture in a water bath to 75°C. One ml of the homogenized sample was combined with two ml of this solution, and the mixture was then cooked in a boiling water bath for 15 min to create a pink hue. The absorbance of the supernatant was assessed using a spectrophotometer (model UNICO UV-2100) at 532 nm after cooling with tap water and centrifuging at 2000×g for 15 min. The TBA value was expressed as mg malondialdehyde/ kg of nugget (Sallam *et al*., 2004).

Determination of biogenic amine (BAs) concentration by HPLC

According to Magwamba *et al*. (2010), five biogenic amines, including histamine (HIS), tyramine (TYR), tryptamine (TRY), putrescine (PUT), and cadaverine (CAD), had been detected in 60 tested samples.

Proximate Composition

The proximate composition of the product was determined following the standard procedure of AOAC (2016).

Statistical analysis

The results are reported as mean±SD. A statistical analysis (Snedecor and Cochran, 1995) for Analysis of Variance (ANOVA) was performed on the data obtained from various trials within each experiment.

RESULTS

The data in Table 2 show the mean value of the microbial load of chicken nuggets over 21 days of storage in which the total plate count (cfu/g) for T1 ranged from 2.28 $\times10^3\pm1.68$ at 0 days to $11.32\times10^3\pm3.58$ at 21 days, for T2 from $1.23\times10^3\pm0.88$ to $1.1\times10^3\pm0.53$, for T3 from $1.08\times10^3\pm0.64$ to $0.048\times10^3\pm0.74$, for T4 from $0.83\times10^3\pm0.42$ to $0.32\times10^3\pm0.49$ and for T5 from $0.55\times10^3\pm0.83$ to $0.023\times10^3\pm0.83$. Regarding the presence of psychrophilic count in examined chicken nuggets was $0.47\times10^3\pm0.73$ to $8.33\times10^3\pm2.46$ for T1, $0.39\times10^3\pm0.08$ to $1.05\times10^3\pm0.17$ for T2, $0.24\times10^3\pm0.63$ to $0.081\times10^3\pm0.32$ for T3, $0.43\times10^3\pm0.49$ to $0.22\times10^3\pm0.18$ for T4, 0.41±0.3 to 0.062±0.2 for T5. The occurrence of yeast and mold in examined samples was found to be $4.8\times10^2\pm2.53$, $2.67\times10^2\pm1.07$, $1.39\times10^2\pm0.92$, $1.56\times10^2\pm1.03$ and $1.52\times10^2\pm0.48$ at 0 days while $9.15\times10^2\pm3.17$, $1.62\times10^2\pm1.41$, $0.61\times10^2\pm0.07$, $1.11\times10^2\pm0.8$ and $0.33\times10^2\pm0.55$ at 21 days for T1, T2, T3.T4, and T5 respectively.

Table 3 demonstrates the mean value of the biogenic amines (BAs) level (mg/kg) in the stored chicken nuggets. Histamine was

detected in all examined groups at 0 days with mean values of 1.9 ±0.08, 0.4±0.01, 1.1±0.01, 1.1±0.01 and 0.3±0.01 mg/kg, respectively, at 7 days 9±0.06 and 0.21±0.02 for T1 and T5, at 15 days 14±1.42 only in T1 and at 21 days 26±3.27 and 0.3±0.08 for T1 and T4. the mean value of Tyramine level (mg/kg) at 0 days to 21 days ranged from 3.9±2.35 to 11±2.35 for T1, 1.4±0.3 to 0.31±1.01 for T2, 1.3±0.3 to 0 for T3, 1.9±0.6 to 0.74±0.04 for T4 and 0.1±0.01 to 0 for T5. Tryptamine was only detected in T1 at 7, 15, and 21 days, with mean values of 0.2±0.04, 0.31±0.01, and 0.5±0.02, respectively. Putrescine detected at 0 day with mean value of 2±0.23, 6.5±1.07, 1.5±0.02, 2.6±0.48, and 1.9±0.08 for T1, T2, T3. T4, and T5 respectively, at 7 days of 6±2.3, 4.7±1.08, 0, 1.4±0.18, and 0.35±0.01, at 15 days of 9±2.88 and 3.2±0.78 for T1 and T2 only, and at 21 days of 13±3.04, 4±1.1 and 0.5±0.03 for

Table 2. Mean value of microbial load of chicken nuggets during storage.

Microbial count	Treatment	Storage period (days)			
		0	7	15	21
Total plate count (cfu/g x 10^3)	T1	2.28±1.68^a	4.62±1.73^a	7.54±2.06^a	11.32±3.58^a
	T2	1.23±0.88^b	1.04±0.51^b	0.86±0.48^b	1.1±0.53^b
	T3	1.08±0.64^b	0.88±0.74^c	0.053±0.63^c	0.048±0.74^c
	T4	0.83±0.42^c	0.68±0.49^c	0.056±0.37^c	0.32±0.49^b
	T5	0.55±0.83^d	0.35±0.83^c	0.027±0.42^d	0.023±0.83^c
Psychrophilic count (cfu/g x 10^3)	T1	0.47±0.73^a	3.23±1.53^a	5.17±1.8^a	8.33±2.46^a
	T2	0.39±0.08^b	0.31±0.15^b	0.28±0.17^b	1.05±0.17^b
	T3	0.24±0.63^b	0.13±0.46^c	0.087±0.66^c	0.081±0.32^c
	T4	0.43±0.49^c	0.38±0.56^b	0.18±0.6^b	0.22±0.18^b
	T5	0.41±0.3^c	0.33±0.73^b	0.065±0.12^c	0.062±0.2^c
Yeast and mold count (cfu/g x 10^2)	T1	4.8±2.53^a	6.32±2.33^a	7.84±2.7^a	9.15±3.17^a
	T2	2.67±1.07^b	1.84±1.5^b	1.33±1.7^b	1.62±1.41^b
	T3	1.39±0.92^c	0.92±0.63^c	0.64±0.04^c	0.61±0.07^c
	T4	1.56±1.03^c	1.23±0.71^b	1.07±0.11^b	1.11±0.8^b
	T5	1.52±0.48^c	0.76±0.52^d	0.38±0.22^d	0.33±0.55^d

T1: Control samples, T2: Samples treated with *Bacillus clausii* 1%, T3: Samples treated *Bacillus clausii* 3%, T4: Samples treated with *Saccharomyces cerevisiae* 1%, T5: Samples treated with *Saccharomyces cerevisiae* 3%. Means in the same column with varying superscripts are significantly different at p≤ 0.05 levels.

Table 3. Mean value (±SD) of biogenic amines level (mg/kg) in the stored chicken nuggets.

Biogenic amines	Treatment	Storage period (days)			
		0	7	15	21
Histamine	T1	1.9 ±0.08^a	9.0±0.06^a	14.0±1.42^a	26.0±3.27^a
	T2	0.4±0.01^b	0	0	0
	T3	1.1±0.01^c	0	0	0
	T4	1.1±0.01^c	0	0	0.3±0.08^b
	T5	0.3±0.01^b	0.21±0.02^b	0	0
Tyramine	T1	3.9±2.35^a	6.32±1.2^a	8.23±1.02^a	11.0±2.35^a
	T2	1.4±0.3^b	0.8±0.03^b	0.5±0.05^b	0.31±1.01^b
	T3	1.3±0.3^b	0.5±0.08^b	0	0
	T4	1.9±0.6^b	0	0	0.74±0.04^b
	T5	0.1±0.01^c	0	0	0
Tryptamine	T1	0^a	0.2±0.04^a	0.31±0.01^a	0.5±0.02^a
	T2	0	0	0	0
	T3	0	0	0	0
	T4	0	0	0	0
	T5	0	0	0	0
Putrescine	T1	2.0±0.23^a	6.0±2.3^a	9.0±2.88^a	13.0±3.04^a
	T2	6.5±1.07^b	4.7±1.08^b	3.2±0.78^b	4.0±1.1^b
	T3	1.5±0.02^c	0	0	0.5±0.03^c
	T4	2.6±0.48^a	1.4±0.18^c	0	0
	T5	1.9±0.08^a	0.35±0.01^c	0	0
Cadaverine	T1	2.0±0.23^a	12.0±2.38^a	16.0±2.46^a	22.0±3.73^a
	T2	1.3±0.51^b	0	0	0.2±0.01^b
	T3	0	0	0	0
	T4	2.5±0.42^a	1.44±0.25^b	0	0.37±0.05^b
	T5	1.9±0.08^a	0.4±0.01^c	0	0

T1: control samples, T2: samples treated with Bacillus clausii 1%, T3: samples treated with Bacillus clausii 3%, T4: samples treated with *Saccharomyces cerevisae* 1%, T5: samples treated with *Saccharomyces cerevisae* 3%. Means in the same column with varying superscripts are significantly different at p≤ 0.05 levels.

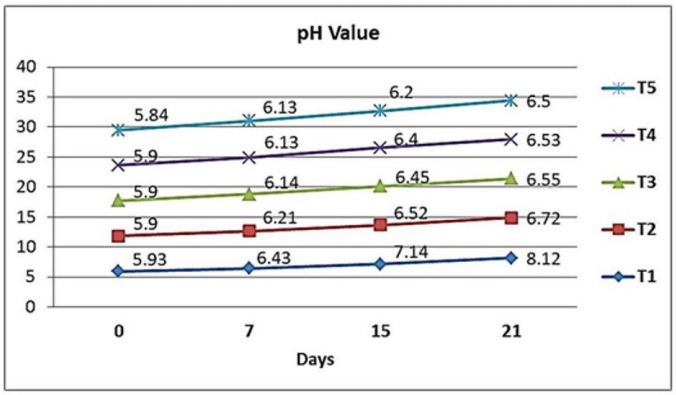

Fig. 1. pH value of chicken nuggets during the storage. T1: control samples, T2: samples treated with *Bacillus clausii* 1%, T3: samples treated with *Bacillus clausii* 3%, T4: samples treated with *Saccharomyces cerevisiae* 1%, T5: samples treated with *Saccharomyces cerevisiae* 3%.

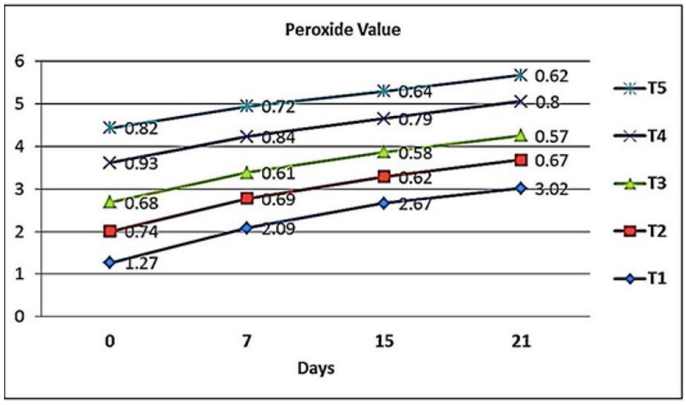

Fig. 2. Peroxide value (nmol/g) of chicken nuggets during the storage. T1: control samples, T2: samples treated with *Bacillus clausii* 1%, T3: samples treated with *Bacillus clausii* 3%, T4: samples treated with *Saccharomyces cerevisiae* 1%, T5: samples treated with *Saccharomyces cerevisiae* 3%.

T1, T2, and T3 only. Cadaverine was detected in T1 over 21 days with mean values of 2 ±0.23, 12±2.38, 16±2.46, and 22±3.73 respectively, for T2 detected only at 0 days and 21 days with a mean of 1.3±0.51 and 0.2±0.01, for T3 not detected all over the period, for T4 only at 0 days and 21 days with a mean of 1.5±0.42 and 0.37±0.05 and T5 detected only at 0 day with mean of 1.21±0.08.

Table 4 shows the approximate composition of a chicken nugget formulated with various *Bacillus clausii* and *Saccharomyces cerevisiae* levels during storage.

The data presented in Figure 1 shows that the mean pH value of chicken nuggets during storage was 5.93, 5.9, 5.9, 5.9, and 5.84 at 0 days, 6.43, 6.21, 6.14, 6.13, and 6.13 at 7 days, 7.14, 6.52, 6.45, 6.4, and 6.2 at 15 days, and 8.12, 6.72, 6.55, 6.53, and 6.5 at 21 days for T1, T2, T3, T4, and T5. The pH value of chicken nuggets during storage was insignificant ($p \geq 0.05$) for the experimental treatment compared with the control one, with significant differences between the examined samples at $p \leq 0.05$.

Figure 2 reported that the mean peroxide value (nmol/g) of

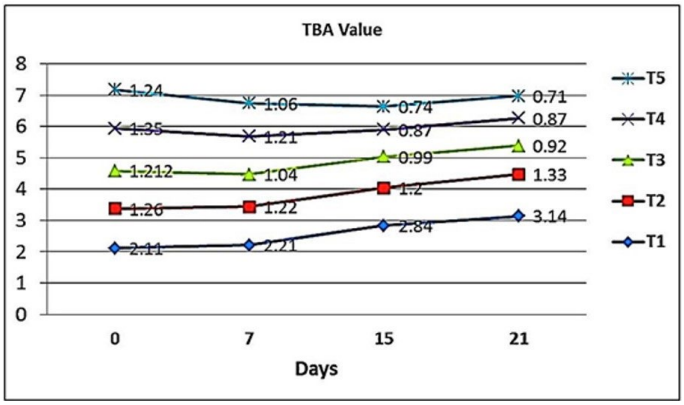

Fig. 3. TBA value (nmol/g) of chicken nuggets during the storage. T1: control samples, T2: samples treated with *Bacillus clausii* 1%, T3: samples treated with *Bacillus clausii* 3%, T4: samples treated with *Saccharomyces cerevisiae* 1%, T5: samples treated with *Saccharomyces cerevisiae* 3%.

Table 4. Mean value (±SD) of proximate composition (%) of the stored chicken nuggets.

Biogenic amines	Treatment	Storage period (days)			
		0	7	15	21
Moisture content	T1	66.36±1.6^{a}	67.82±1.8^{a}	69.42±2.01^{a}	71.31±2.34^{a}
	T2	66.22±1.3^{a}	66.37±1.52^{b}	67.53±1.27^{a}	68.21±2.65^{b}
	T3	64.18±1.52^{b}	65.38±1.22^{c}	66.63±1.31^{b}	67.73±2.62^{c}
	T4	65.82±1.71^{a}	66.12±1.01^{b}	67.12±1.32^{a}	68.12±2.11^{b}
	T5	64.38±1.63^{b}	64.88±1.42^{c}	65.26±1.7^{b}	66.58±2.54^{c}
Protein	T1	17.75±1.4^{a}	17.42±1.21^{a}	15.18±1.81^{a}	11.64±2.04^{a}
	T2	17.84±1.32^{a}	17.44±1.51^{a}	17.37±1.36^{b}	15.38±1.71^{b}
	T3	18.95±2.2^{b}	18.77±1.4^{b}	18.45±1.09^{c}	17.78±1.63^{c}
	T4	18.21±2.33^{b}	18.04±1.61^{c}	17.87±1.52^{b}	16.54±1.61^{c}
	T5	18.25±1.72^{b}	18.23±1.44^{b}	18.16±1.72^{c}	17.44±1.7^{c}
Fat	T1	12.20±0.63^{a}	10.84±1.33^{a}	9.38±1.83^{a}	8.38±1.53^{a}
	T2	11.78±0.14^{a}	11.6±1.05^{b}	10.48±1.2^{b}	9.48±1.62^{b}
	T3	11.41±0.41^{a}	11.41±0.66^{b}	10.33±0.5^{b}	9.33±0.18^{b}
	T4	11.66±0.33^{a}	11.58±0.72^{b}	11.15±1.7^{b}	9.15±1.3^{b}
	T5	10.83±0.51^{b}	10.62±1.21^{c}	10.08±1.35^{b}	10.08±1.5^{c}
Ash	T1	1.67±0.2^{a}	1.34±0.05^{a}	1.29±0.05^{a}	1.07±0.07^{a}
	T2	1.83±0.04^{a}	1.66±0.01^{b}	1.52±0.03^{b}	1.02±0.04^{a}
	T3	1.47±0.2^{b}	1.32±0.01^{a}	1.22±0.06^{a}	1.12±0.08^{b}
	T4	1.76±0.05^{a}	1.58±0.03^{b}	1.4±0.01^{b}	1.19±0.01^{b}
	T5	1.65±0.05^{a}	1.44±0.08^{b}	1.37±0.05^{b}	1.06±0.01^{a}

T1: control samples, T2: samples treated with *Bacillus clausii* 1%, T3: samples treated with *Bacillus clausii* 3%, T4: samples treated with *Saccharomyces cerevisiae* 1%, T5: samples treated with *Saccharomyces cerevisiae* 3%. Means in the same column with varying superscripts are significantly different at $p \leq 0.05$ levels.

chicken nuggets for T1, T2, T3.T4, and T5 was 1.27, 0.74, 0.68, 0.93, and 0.82 at 0 days, 2.09, 0.69, 0.61, 0.84, and 0.72 at 7 days, 2.67, 0.62, 0.58, 0.79, and 0.64 at 15 days and 3.02, 0.67, 0.57, 0.8, and 0.62 at 21 days.

Figure 3 stated the mean Thiobarbituric acid (TBA) value (mg malondialdehyde/kg) of chicken nuggets at 0 days was 2.11, 1.26, 1.21, 1.35, and 1.24, at 7 days was 2.21, 1.22, 1.04, 1.21, and 1.06, at 15 days was 2.84, 1.2, 0.99, 0.87, and 0.74 and at 21 days was 3.14, 1.33, 0.92, 0.87, and 0.71 for T1, T2, T3.T4, and T5.

DISCUSSION

Developing value-added products like chicken nuggets is the best strategy to boost poultry meat consumption. These ready-to-fry and served pre-processed products are gaining popularity in the consumer market. This rise in product quality raises the marketability of chicken products. Processing, raw materials, and ingredient factors, either from nutritional value or general customer appeal, substantially impact the quality of the nuggets.

In all samples evaluated, the experimentally treated groups T2, T3, T4, and T5 had a low microbial count (Table 2). Specifically, the treated group with *Bacillus clausii* 3% and *Saccharomyces cerevisiae* 3% had significantly lower ($p \leq 0.05$) microbial count, followed by the treated group with *Bacillus clausii* 1% and *Saccharomyces cerevisiae* 1%. The microbial loads decrease over storage days, especially on days 7 and 15, followed by an increase on day 21 for T2 and T4. Additionally, a substantial variation in TPC was seen, psychrophilic count, and yeast and mold count in chicken nuggets ($p < .05$). The initial samples microbial load was greatly influenced by the cleanliness conditions of chicken handling and preparation (Moosavi-Nasab *et al*., 2019). The interaction between the type and concentration of probiotics and storage days revealed a similar trend.

These readings were within the normal range for such treatment. They agreed with the findings of Jafari *et al*. (2017), Moosavi-Nasab *et al*. (2019), and Opeyemi *et al*. (2022), who stated that the usage of *Bacillus* probiotics is a remarkable way to produce healthier chicken meat products as functional foodstuffs, as well as a valuable solution for overcoming the constraints related to microbial growth during food processing and storage. Due to immunological stimulation, antibacterial properties, and competitive exclusion, the use of *Bacillus* species spores as probiotic food supplements is spreading widely and relatively quickly (Katsutoshi *et al*., 2011). In contrast, the increasing trend in microbiological count was reported by Sharma *et al*. (2018). Additionally, Opeyemi *et al*. (2002) speculate that the variance may be due to the synthesis of certain metabolites that have altered microbial succession.

The results in Fig. 1 demonstrated that different treatments had different pH values for the manufactured chicken nuggets. All chicken nuggets' pH values gradually increased throughout storage. These readings were within the normal range for such products except the control one (T1), which spoiled on day 21, and they agreed with the data found by Abd-El-Aziz *et al*. (2021) and Silva *et al*. (2021). Meanwhile, the mean pH values of chicken meat nuggets were not affected by different formulations ($p \geq 0.05$), according to Para *et al*. (2015) and Jeswanth *et al*. (2022).

Lipid oxidation, which can impair the activity of proteins and produce product discoloration, off-odor, and off-flavoring, significantly contributes to the deterioration of frozen chicken nuggets (Al-Hijazeen *et al*., 2016). The PA and TBA standards are used to assess the oxidation of meat and meat products. The greater value of PA and TBA indicates that the product is oxidizing. Figures 2 and 3 demonstrate that the interaction between the effects of treatment and storage duration had a significant ($p \leq 0.05$) impact on the PA and TBA of chicken meat nuggets. During the 21-day storage period, the PA and TBA values of the nuggets decreased in the treatments (T2, T3, T4, and T5) but increased in the control treatment (T1). These results demonstrated the formation of hydroperoxides as primary lipid oxidation products in the control group (Hwang *et al*., 2011). However, the PA and TBA values of the morsels increased over time, with the greatest increase occurring on day 21. TBA value is a measure of lipid oxidation in meat products caused by the generation of aldehydes and carbonyls from hydrocarbons, and the rancid flavor is initially noticed in meat products with TBA values between 0.5 and 2.0 (Moosavi-Nasab *et al*., 2019).

In contrast, except for the control group, the current was lower than this range. A similar observation was stated by Mishra *et al*. (2015), Faiz *et al*. (2020), and Jeswanth *et al*. (2022). These findings disagree with Vanitha *et al*., (2015) and Moosavi-Nasab *et al*., (2019). The variation in results could be credited to lipid oxidation and the production of volatile metabolites in the occurrence of oxygen, which is caused by the oxygen permeability of the packaging material (Faiz *et al*., 2020).

BAs are nitrogenous compounds that occur in various food chains at varying concentrations depending on several variables, including the profile of free amino acids, the microbial quality, and the hygienic practices used during food preparation (Martuscelli *et al*., 2021). Meat is often regarded as a key reservoir of BAs owing to its highly gratified amino acids from which they are derived (Ruiz-Capillas and Herrero, 2019). The high concentration of proteins (amino acids) also depends on the nature of meat, which is composed of muscular tissue whose cells are more susceptible to BAs. The significance of biogenic amines stems from their use as a marker of bacteriological quality, freshness, and food degradation, in addition to endangering the public's health (Chaidoutis *et al*., 2019). Also, Esposito *et al*. (2022) stated that the most predominant BAs in chicken meat are tyramine, histamine, and polyamines (spermidine, spermine, cadaverine, and putrescine). The results of this study (Table 3) have not found significant differences among different treatments for HIS, TYR, TRY, PUT, and CAD at days 7, 15, and 21. The concentration of BAs decreased significantly by the storage period in the treatments (T2, T3, T4, and T5) of the nuggets. At the same time, the increase in the BAs was recorded in the control treatment (T1) during the storage period. This agrees with Fraqueza *et al*. (2012) and Esposito *et al*. (2022) noted a consistent decline. Esposito *et al*. (2022) say meat is considered good quality when the BAs result is less than 5 mg kg^{-1}. Then, if BAs values are between 5 and 20 mg kg^{-1}, meat is regarded as acceptable with early spoiling indicators; if BAs findings are between 20 and 50 mg kg^{-1}, samples are considered low quality; and, ultimately, BAs values greater than 50 mg kg^{-1} are associated with being deemed spoiled. Data from this study disclose a high quality of the chicken nuggets treated with probiotics that are highly preserved for at least 21 days of frozen storage.

Factors such as processing techniques, raw materials, and additives considerably impact chicken nuggets' quality and nutritional quality (El-Anany *et al*., 2020). In the current investigation, as the storage period progressed, the corresponding value for moisture content in all control and treatment groups increased linearly at a uniform rate, which could be attributed to the hygroscopic nature of chicken meat (Sarkar *et al*., 2020). The interaction of chicken nugget formulations and storage period substantially impacts the chemical composition of meat ($p \leq 0.05$). The rise in moisture content and the decrease in protein content detected during the investigation could be attributed to the denaturation of chicken meat protein that is related to frozen chicken meats following Sharma *et al*. (2018), Hammad *et al*. (2019), and El-Anany *et al*. (2020). The findings of Jeswanth *et al*. (2022) did not coincide with ours, possibly due to differences in temperature, time, meat kind, and fodder type.

The higher moisture content could be due to water absorption during thawing or degradation of specific meat components, which releases bound water. Furthermore, the changes in fat content identified during frozen storage up to day 21 might be attributed to fat oxidation or hydrolysis. Still, the decrease in protein content reported in the study could be attributed to the

denaturation of beef protein caused by frozen conditions. On the other hand, the deterioration of the ash shown during storage could be ascribed to fat, protein, and water hydrolysis. In addition to clinical data supporting the health benefits of probiotic microorganisms, this aspect is crucial for factories and research centers interested in formulating products with probiotic microorganisms. Consequently, they are an ideal option for developing functional meat-based products, representing a significant portion of the meat industry.

CONCLUSION

The current increase in customer demand for healthy meat products has compelled the industry and scientific-technological community to develop new functional meat products. Bacterial spore-formers and yeast as probiotics are becoming more popular in the food industry. In this context, using probiotics in the formulation has become a key technique for improving meat products and adding higher added value. However, producing probiotic chicken products necessitates strict quality control to generate functional meat products with genuine human health advantages. Furthermore, the additive supplementation of *Bacillus clausii* and *Saccharomyces cerevisiae* improves the shelf life attributes, physicochemical aspects, and microbiological quality of chicken nuggets.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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