

# Microbial quality of cooking butter after addition of curcumin extract during refrigerated storage

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## ABSTRACT

Cooking butter is a popular type of food consumed in Egypt. However, its microbial quality may deteriorate during conventional production methods, potentially leading to health risks. The shelf life of cooking butter can be extended by incorporating natural preservatives. Curcumin extract is one such natural compound known for its antimicrobial properties. The objective of this study was to assess the microbial quality of cooking butter stored at  $5\pm 1^{\circ}\text{C}$  for 30 days after the addition of curcumin extract at concentrations of 2% and 4% (v/w). The butter samples were analyzed for the presence of coliforms, fecal coliforms, *E. coli*, psychrotrophic bacteria, lipolytic bacteria, enterococci, yeasts, and molds at several intervals, including the initial time, the second day, and then weekly until the end of the storage period. The results revealed that both 2% and 4% curcumin extract significantly reduced all investigated microbial counts during the storage period from the first to the last week, with a more pronounced reduction observed at the 4% concentration. The sensory evaluation confirmed that the curcumin-enriched butter exhibited desirable attributes. These findings indicate that curcumin extract is a potent antimicrobial agent, capable of reducing microbial contamination in cooking butter during refrigerated storage, offering a safer alternative to artificial preservatives.

## Introduction

Butter is an important component of the human diet, providing a variety of essential nutrients. Also, it contains milk fat globule membrane, which is abundant in phospholipids that play a critical role in various physiological processes. These processes include cell growth, regulation of apoptosis, signal transduction, blood clotting, and neurotransmission (Pettus *et al.*, 2004; Tvřicka *et al.*, 2011). Furthermore, butter contributes significantly to the organoleptic properties of food, giving a desirable aroma during cooking (Sert and Mercan, 2020).

Cooking butter is traditionally produced in rural areas using conventional methods, including a churning process. The lack of advanced technology and hygienic protocols in production, packaging, storage, and transportation may result in a decline in butter quality, posing potential hazards to human health (Koca *et al.*, 2010). The butter provides an ideal medium for microbial growth due to its composition, which includes approximately 20% water, proteins, and milk sugars (Catsberg and Kempen-van Dommelen, 2013).

Total coliforms are commonly used as indicators of inadequate hygiene during butter production (Tofangszan *et al.*, 2009). Similarly, the presence of *E. coli* is frequently associated with sanitary concerns, particularly when contamination arises from exposure to fecal matter. This can occur if raw milk is contaminated with fecal material or if traces of fecal coliforms are introduced by individuals involved in the manufacturing process (Meshref, 2010). Certain virulent strains of *E. coli* may lead to several health complications such as hemolytic uremic syndrome, meningitis in neonates, gastrointestinal disturbance, diarrheal diseases, sepsis and urinary tract infections (Barrett *et al.*, 2005).

Psychrotrophic bacteria can thrive in cold temperatures during butter storage, leading to rancidity and physical defects (Catsberg and Kempen-van Dommelen, 2013). Also, these bacteria can deteriorate milk products by releasing proteolytic and lipolytic enzymes, some of which remain

active after milk heat treatment, altering their nutritional value and storage stability (Khalifa and Shata, 2018). Lipolysis is the process of breaking down milk fats into free fatty acids and glycerides by enzymatic action. The formation of free fatty acids has been associated with adverse health effects, such as increased risk of cardiovascular diseases and arteriosclerosis (Deeth, 2020).

Continuous monitoring of enterococci in dairy products is essential to ensure their safety and suitability for human consumption (Martín *et al.*, 2010). Fungal spoilage of dairy products can occur at any stage, from production on the dairy farm to the point of delivery to consumers. In dairy processing plants, contamination by yeasts and molds can arise after milk pasteurization, as these fungi are generally not heat-resistant. Airborne fungal spores can easily disseminate throughout the environment where dairy products are produced (Kure *et al.*, 2004).

In recent years, the fortification of dairy products with food additives has gained attention to extend their shelf life and enhance their physicochemical and microbial properties (Ansari and Kumar, 2012). Among these additives, herbs and spices are commonly used to improve organoleptic characteristics and serve as antimicrobial agents against foodborne pathogens (Abdollahzadeh *et al.*, 2014). Curcumin, which is approved by the Codex Alimentarius Commission, is widely utilized as a safe preservative for coloring and flavoring various dairy products (Chaves *et al.*, 2021). Turmeric, the source of curcumin, contains bioactive compounds known as curcuminoids, which are considered beneficial when consumed in amounts up to 8 grams per day, as approved by the Food and Drug Administration (Rahmat *et al.*, 2021).

The phenolic compounds present in turmeric contribute to product quality by stabilizing acidity and reducing oxidative stress. Additionally, these compounds are effective in inhibiting the growth of various microorganisms, including coliforms, fungi, *E. coli*, *Klebsiella*, and *Staphylococcus aureus* (Doosh *et al.*, 2022). In butter and other milk-based products, curcumin prevents the proliferation of pathogenic bacteria due to its

methoxyl and hydroxyl group content (Han and Yang, 2005). This study aimed to evaluate the microbial characteristics of cooking butter after the addition of curcumin extract at different intervals during refrigerated storage.

## Materials and methods

### Material sources

Curcumin powder was obtained as a reference material from the National Research Centre, Cairo, Egypt. All culture media were purchased from Himedia Company (India). Chemicals and reagents were supplied by Sigma-Aldrich Company (Egypt). All materials were of analytical grade.

### Preparation of curcumin extract

Curcumin extraction was performed by mixing curcumin powder with distilled water and boiling the mixture for 6 h. After boiling, the extract was filtered and concentrated using spray drying. The resulting concentrated extract was then stored at 4°C until used (Sadashiva *et al.*, 2019).

### Microbial evaluation of cooking butter samples

Under aseptic conditions, 30 random samples of cooking butter were collected from various locations in Assiut City, Egypt. The samples were promptly transported to the laboratory of the Food Hygiene, Safety, and Technology Department at the Faculty of Veterinary Medicine, Assiut University, Egypt, for analysis of their microbial quality.

### Preparation of incorporated cooking butter samples with curcumin extract

The cooking butter sample was divided into 3 equal parts (A, B and C). All parts were thoroughly mixed in a water bath (40°C), during mixing adding to parts A and B, 2% and 4% curcumin extract, respectively, and part C was kept as a control (without adding curcumin extract). Test and control samples were evaluated for sensory characteristics. The samples were stored in a refrigerator (5±1°C) for 30 days. Microbial evaluation was performed on all samples in triplicate at the following intervals: initial time, second day, and after the first, second, third, and fourth weeks of storage.

### Microbiological examination

The initial suspension of the butter samples and their subsequent decimal dilutions were prepared using 0.1% sterile buffered peptone water (APHA, 1992). For the determination of total coliforms and fecal coliforms, violet red bile agar was used, with incubation at 32°C and 45°C, respectively, for 24 h (APHA, 2001). *E. coli* enumeration was performed using eosin methylene blue agar, incubated at 30°C for 48 h (ISO, 1995),

and followed by confirmation through biochemical tests depending on Cheesbrough (1981). Psychrotrophic bacteria were enumerated using plate count agar with an incubation period of 10 days at 7°C±1°C according to ISO (2019). Lipolytic bacteria were counted using the spread plate technique on tributyrin agar plates, with incubation at 37°C for 24–72 h (Sagar *et al.*, 2013). Enterococci counts were determined using kanamycin esculin agar, incubated at 37°C for 48 h (Gobbetti *et al.*, 1999). Yeast and mold counts were carried out by the method of Tantaoui-Elaraki *et al.* (1983) using sabouraud dextrose agar, with incubation at 30°C for 3 days. All microbial counts were expressed as log CFU/g.

### Sensory evaluation

The control butter and the fortified butter samples containing 2% and 4% curcumin extract were subjected to sensory evaluation for attributes including odor, taste, color, body & texture, and overall acceptability, using the method described by Larmond (1977), based on a 9-point hedonic scale.

### Statistical analysis

The results were statistically analyzed using GraphPad Prism 5 (version 5.01). Data are presented as mean ± standard error (SE). A one-way analysis of variance (ANOVA) followed by Tukey's comparison test was performed to assess significant differences ( $p < 0.05$ ) among the different types of cooking butter for each microbial count.

## Results

### Microbial evaluation of cooking butter samples

The statistical analytical results of microorganisms count in the positive examined cooking butter samples (Table 1) showed that the mean values of coliform bacteria, fecal coliforms, *E. coli*, psychrotrophic bacteria, lipolytic bacteria, enterococci, yeasts, and molds were 4.14±0.29, 5.40±0.03, 4.35±0.78, 3.44±0.14, 3.43±0.20, 4.39±0.24, 3.46±0.23, 3.17±0.22 log CFU/g, respectively.

### Effect of curcumin extract on microbial quality of cooking butter

As indicated in Table 2, the coliform count in fresh butter was 5.61±0.21 log CFU/g, which increased to 5.91±0.23 log CFU/g at the last week of storage. In contrast, butter enriched with 2% curcumin extract showed a marked decline to 1.60±0.05 log CFU/g, while no coliforms were detected in samples with 4% curcumin by the final week. Similarly, the fecal coliform count in control butter at the start of storage was 5.38±0.10 log CFU/g, which increased to 5.77±0.11 log CFU/g at the finish of storage. However, no fecal coliform contamination was detected in the butter with 2% curcumin extract at the final of storage, and no con-

Table 1. Statistical analytical results of microorganisms count in the positive examined cooking butter samples.

Microorganisms	Positive samples		Count (log CFU/g)		
	No. /30	%	Min.	Max.	Mean ± SE
Coliforms	7	23.33	2.03	5.79	4.14 ± 0.29
Fecal coliforms	3	10	5.31	5.62	5.40 ± 0.03
<i>E. coli</i>	2	6.67	3.8	4.91	4.35 ± 0.78
Psychrotrophic	16	53.33	2.05	5.88	3.44 ± 0.14
Lipolytic	22	73.33	1.98	5.93	3.43 ± 0.20
Enterococci	7	23.33	3.04	5.88	4.39 ± 0.24
Yeasts	15	50	2	5.92	3.46 ± 0.23
Molds	19	63.33	1.97	5.87	3.17 ± 0.22

Data are presented as Mean±standard error (SE)

tamination was observed from the third to the fourth week in the butter with 4% curcumin extract. The *E. coli* count in the control butter gradually increased during the storage period, ultimately reaching 4.28±0.18 log CFU/g. Conversely, *E. coli* was undetectable in the butter samples with 2 and 4% curcumin extract from the third to the fourth week and from the second to the fourth week, respectively.

Results in Table 3 cleared that the psychrotrophic count in the control butter remained relatively stable throughout the storage period, ranging from 6.70±0.10 to 6.79±0.13 log CFU/g. Otherwise, butter samples fortified with 2% curcumin extract showed a significant decrease in the psychrotrophic count, which dropped to 2.31±0.05 log CFU/g at the end of storage. The reduction was more pronounced in butter samples with 4% curcumin extract, where the psychrotrophic reached a mean value of 1.32±0.17 log CFU/g. The initial lipolytic count in the control butter was 5.81±0.18 log CFU/g, increasing slightly to 5.91±0.29 log CFU/g by the last week of storage. While, the lipolytic count in butter fortified with 2 and 4% curcumin extract reached a mean value of 1.61±0.10 and 1.01±0.02 log CFU/g, respectively at the last week of storage (Table 3).

The enterococci count in the control butter showed no significant changes during the storage period, remaining between 5.85±0.23 and 5.81±0.15 log CFU/g. On the opposite side, butter samples fortified with 2% curcumin extract had a mean value of 1.70±0.18 log CFU/g and butter fortified with 4% curcumin extract exhibited a mean value of 1.32±0.16 log CFU/g (Table 3).

According to Table 4, the yeast and mold counts in the control butter samples increased slightly by the end of the storage period, reaching 5.88±0.27 and 5.93±0.23 log CFU/g, respectively. Otherwise, butter samples with 2% curcumin extract had a mean yeast count of 1.81±0.17 log CFU/g and a mean mold count of 1.84±0.18 log CFU/g. Moreover, yeasts or molds were undetectable in the butter samples containing 4% curcumin extract by the fourth week of storage.

*Sensory evaluation of cooking butter samples after addition of curcumin extract*

Sensory evaluation of the control butter and the butter treated with 4% curcumin extract (Figure 1) illustrated that both samples received high sensory scores, with ratings of 8 and 7 across all evaluated attributes, respectively. In contrast, the butter containing 2% curcumin extract scored 8 for odor, 7 for taste and body & texture, and 6 for color.

**Discussion**

Total coliforms are considered a critical indicator of cleanliness when evaluating the microbiological quality of butter (Bereda *et al.*, 2014). The data provided in Table 1 revealed that 23.33% of the analyzed butter samples were positive for coliform bacteria. These results suggest that the majority of the examined butter samples (76.67%) complied with the

Table 2. Effect of curcumin extract (CE) addition on coliforms, fecal coliforms, and *E. coli* counts (log CFU/g) in cooking butter samples.

Storage period	Coliforms			Fecal coliforms			<i>E. coli</i>		
	Control	2% CE	4% CE	Control	2% CE	4% CE	Control	2% CE	4% CE
Initial time	5.61±0.21 <sup>a</sup>	5.63±0.49 <sup>a</sup>	5.60±0.60 <sup>a</sup>	5.38±0.10 <sup>a</sup>	5.41±0.18 <sup>a</sup>	5.40±0.10 <sup>a</sup>	2.83±0.18 <sup>a</sup>	2.84±0.11 <sup>a</sup>	2.82±0.16 <sup>a</sup>
2 <sup>nd</sup> day	5.71±0.11 <sup>a</sup>	5.59±0.11 <sup>a</sup>	5.58±0.10 <sup>a</sup>	5.52±0.12 <sup>a</sup>	5.29±0.18 <sup>a</sup>	5.01±0.19 <sup>a</sup>	2.93±0.18 <sup>a</sup>	2.80±0.17 <sup>a</sup>	2.58±0.11 <sup>a</sup>
1 <sup>st</sup> week	5.58±0.17 <sup>a</sup>	4.52±0.11 <sup>ab</sup>	3.72±0.12 <sup>c</sup>	5.62±0.18 <sup>a</sup>	3.81±0.16 <sup>b</sup>	2.93±0.17 <sup>c</sup>	3.29±0.06 <sup>a</sup>	1.90±0.08 <sup>b</sup>	1.72±0.11 <sup>b</sup>
2 <sup>nd</sup> week	5.84±0.17 <sup>a</sup>	3.71±0.18 <sup>b</sup>	2.80±0.13 <sup>c</sup>	5.48±0.22 <sup>a</sup>	2.82±0.13 <sup>b</sup>	1.68±0.12 <sup>c</sup>	3.70±0.23 <sup>a</sup>	1.48±0.16 <sup>b</sup>	0.00 <sup>c</sup>
3 <sup>rd</sup> week	5.90±0.12 <sup>a</sup>	2.79±0.15 <sup>b</sup>	1.72±0.15 <sup>c</sup>	5.65±0.18 <sup>a</sup>	1.61±0.16 <sup>b</sup>	0.00 <sup>c</sup>	3.90±0.10 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>
4 <sup>th</sup> week	5.91±0.23 <sup>a</sup>	1.60±0.05 <sup>b</sup>	0.00 <sup>c</sup>	5.77±0.11 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>	4.28±0.18 <sup>a</sup>	0.00 <sup>b</sup>	0.00 <sup>b</sup>

Data are presented as mean ± standard error (SE). Differences between means marked with different superscript letters in the same row are significant and with the same letters are not significant for each microbial count separately.

Table 3. Effect of the addition of curcumin extract (CE) on psychrotrophic, lipolytic and enterococci counts (log CFU/g) in cooking butter samples.

Storage period	Psychrotrophic			Lipolytic			Enterococci		
	Control	2% CE	4% CE	Control	2% CE	4% CE	Control	2% CE	4% CE
Initial time	6.70±0.10 <sup>a</sup>	6.71±0.06 <sup>a</sup>	6.72±0.18 <sup>a</sup>	5.81±0.18 <sup>a</sup>	5.79±0.04 <sup>a</sup>	5.81±0.23 <sup>a</sup>	5.85±0.23 <sup>a</sup>	5.81±0.23 <sup>a</sup>	5.80±0.18 <sup>a</sup>
2 <sup>nd</sup> day	6.90±0.23 <sup>a</sup>	6.81±0.16 <sup>a</sup>	6.70±0.18 <sup>a</sup>	5.82±0.04 <sup>a</sup>	5.69±0.11 <sup>a</sup>	5.72±0.2 <sup>a</sup>	5.69±0.10 <sup>a</sup>	5.63±0.18 <sup>a</sup>	5.60±0.18 <sup>a</sup>
1 <sup>st</sup> week	6.80±0.05 <sup>a</sup>	5.35±0.15 <sup>b</sup>	4.84±0.14 <sup>b</sup>	5.69±0.17 <sup>a</sup>	4.71±0.30 <sup>b</sup>	4.32±0.17 <sup>c</sup>	5.83±0.21 <sup>a</sup>	4.70±0.16 <sup>b</sup>	3.89±0.24 <sup>c</sup>
2 <sup>nd</sup> week	6.90±0.19 <sup>a</sup>	4.49±0.16 <sup>b</sup>	3.71±0.11 <sup>c</sup>	5.89±0.18 <sup>a</sup>	3.60±0.02 <sup>b</sup>	2.90±0.08 <sup>c</sup>	5.80±0.13 <sup>a</sup>	3.33±0.09 <sup>b</sup>	2.81±0.22 <sup>b</sup>
3 <sup>rd</sup> week	6.91±0.17 <sup>a</sup>	3.50±0.12 <sup>b</sup>	2.30±0.11 <sup>c</sup>	5.93±0.11 <sup>a</sup>	2.73±0.11 <sup>b</sup>	1.79±0.13 <sup>c</sup>	5.71±0.19 <sup>a</sup>	2.32±0.16 <sup>b</sup>	1.79±0.12 <sup>b</sup>
4 <sup>th</sup> week	6.79±0.13 <sup>a</sup>	2.31±0.05 <sup>b</sup>	1.32±0.17 <sup>c</sup>	5.91±0.29 <sup>a</sup>	1.61±0.10 <sup>b</sup>	1.01±0.02 <sup>b</sup>	5.81±0.15 <sup>a</sup>	1.70±0.18 <sup>b</sup>	1.32±0.16 <sup>b</sup>

Data are presented as mean ± standard error (SE). Differences between means marked with different superscript letters in the same row are significant and with the same letters are not significant for each microbial count separately.

Table 4. Effect of the addition of curcumin extract (CE) on yeasts and molds counts (log CFU/g) in cooking butter samples.

Storage period	Yeasts			Molds		
	Control	2% CE	4% CE	Control	2% CE	4% CE
Initial time	5.74±0.09 <sup>a</sup>	5.72±0.12 <sup>a</sup>	5.75±0.20 <sup>a</sup>	5.81±0.06 <sup>a</sup>	5.81±0.18 <sup>a</sup>	5.80±0.23 <sup>a</sup>
2 <sup>nd</sup> day	5.81±0.24 <sup>a</sup>	5.70±0.16 <sup>a</sup>	5.69±0.11 <sup>a</sup>	5.79±0.20 <sup>a</sup>	5.78±0.07 <sup>a</sup>	5.59±0.16 <sup>a</sup>
1 <sup>st</sup> week	5.91±0.12 <sup>a</sup>	4.54±0.13 <sup>b</sup>	3.90±0.18 <sup>c</sup>	5.82±0.23 <sup>a</sup>	4.54±0.14 <sup>b</sup>	3.83±0.07 <sup>c</sup>
2 <sup>nd</sup> week	5.89±0.22 <sup>a</sup>	3.61±0.16 <sup>b</sup>	2.60±0.11 <sup>c</sup>	5.91±0.12 <sup>a</sup>	3.52±0.22 <sup>b</sup>	2.81±0.24 <sup>b</sup>
3 <sup>rd</sup> week	5.86±0.15 <sup>a</sup>	2.72±0.12 <sup>b</sup>	1.82±0.23 <sup>c</sup>	5.89±0.05 <sup>a</sup>	2.32±0.12 <sup>b</sup>	1.53±0.28 <sup>c</sup>
4 <sup>th</sup> week	5.88±0.27 <sup>a</sup>	1.81±0.17 <sup>b</sup>	0.00 <sup>c</sup>	5.93±0.23 <sup>a</sup>	1.84±0.18 <sup>b</sup>	0.00 <sup>c</sup>

Data are presented as mean ± standard error (SE). Differences between means marked with different superscript letters in the same row are significant and with the same letters are not significant for each microbial count separately.

Egyptian Standards (2005), which stipulate that the coliform count in butter must be below 10 CFU/g. The high levels of microorganisms observed during the traditional production of dairy products are often attributed to insufficient knowledge of preventive measures that should be implemented by producers and distributors. Lower coliform counts were reported by Hassanzadazar *et al.* (2017) and Gebril *et al.* (2021).

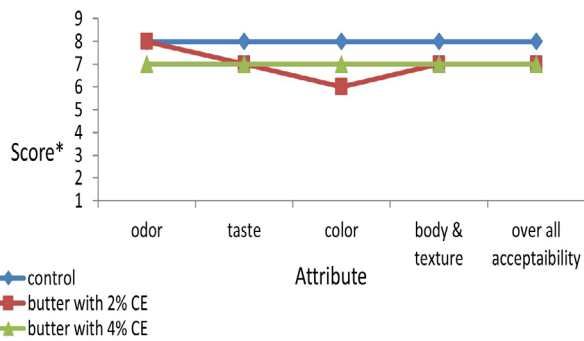


Figure 1. Sensory evaluation of control butter sample and butter enriched with curcumin extract (CE).

\*: 9= extremely liked, 8= very much liked, 7= moderately liked, 6= slightly liked, 5=neither liked nor disliked, 4= slightly disliked, 3= moderately disliked, 2= very much disliked, 1= extremely disliked.

In the same table, 3 out of 30 samples (10%) were contaminated with fecal coliforms, which are considered unacceptable according to Egyptian Standards (2005), as butter must be free of fecal coliforms. Moreover, 2 out of 30 samples (6.67%) were positive for *E. coli*. These positive samples failed to meet the requirements of Egyptian Standards (2005). In contrast, Dervisoglu *et al.* (2013) reported a mean *E. coli* value of 2.29±0.82 log CFU/g. The presence of *E. coli* contamination in butter is attributed to fecal contamination in the milk used for production, which can occur when the teats and udders of cattle come into contact with feces during milking (Ribeiro *et al.*, 2019).

Also, Table 1 cleared that 53.33% of butter samples were contaminated with psychrotrophic bacteria. On the other hand, the mean psychrotrophic bacteria count in the investigated butter samples was 9.30×10<sup>4</sup> CFU/g (El-Bassiony and Khalifa, 2022). While Hassan *et al.* (2022) reported a value of 3.2 × 10<sup>5</sup> CFU/g. Lipolytic bacteria was found in 73.33% of the cooking butter samples (Table 1). The detection of enterococci in dairy products may result from insufficient pasteurization or post-pasteurization contamination, both of which could elevate the risk of foodborne illness (Giraffa, 2002).

The environment serves as the primary source of contamination with yeasts and molds in butter produced through artisan methods (Ghasemloy Incheh *et al.*, 2017). Low water activity in foods promotes the growth of yeasts and molds, which can lead to the formation of mycotoxins, some of which, such as aflatoxins, are known to be carcinogenic (Bullerman, 1981). The result summarized in Table 1 indicated that 50% and 63.33% of cooking butter samples were positive for yeasts and molds, respectively. These values are inconsistent with Egyptian Standards (2005), as butter should be free from yeasts and molds.

The microbial assessment of the cooking butter samples in this study underscores the need to fortify cooking butter with curcumin as a natural antimicrobial agent. Curcumin, a yellow-orange pigment soluble in oil, enhances food flavor and helps preserve its quality. Safety evaluation studies have shown no toxic effects from the use of curcumin, as it is well tolerated even at very high doses (Vinha *et al.*, 2018; Rathore *et al.*, 2020). In this study, curcumin extract concentrations of 2 and 4% were used, falling within the 1-5% range that has been identified as optimal for inhibiting bacterial growth (Shelef *et al.*, 1980; Lourenço *et al.*, 2013).

The organoleptic evaluation of the butter samples following the incorporation of curcumin extract (Figure 1) revealed that butter containing 2 and 4% curcumin extract was deemed acceptable, receiving a score of

7 (moderately liked) for overall acceptability. The addition of curcumin to dairy products may enhance their functional and nutritional attributes, as it significantly influences the fermentation processes by moderating acidity. Moreover, curcumin positively impacts the sensory characteristics of these dairy products (Zheng *et al.*, 2019; Buniowska-Olejnik *et al.*, 2023).

The results outlined in Table 2 and Figure 2 indicated that the coliform count in butter with 2 and 4% curcumin extract decreased by 72.93 and 100%, respectively by the end of the storage period. Also, fecal coliform count achieved a 100% reduction in the butter with 2% curcumin extract at the final of storage, and in the butter with 4% curcumin extract from the third to the fourth week of storage. Moreover, butter samples with 2 and 4% curcumin extract decreased *E. coli* count by 100% from the third to the fourth week and from the second to the fourth week of storage, respectively. However, Wang *et al.* (2009) demonstrated that curcumin exhibited the least inhibitory effect on *E. coli*. Significant differences ( $p < 0.05$ ) were observed between the butter samples with 2 and 4% curcumin extract and the control samples in terms of coliform, fecal coliform, and *E. coli* counts from the first to the last week of storage.

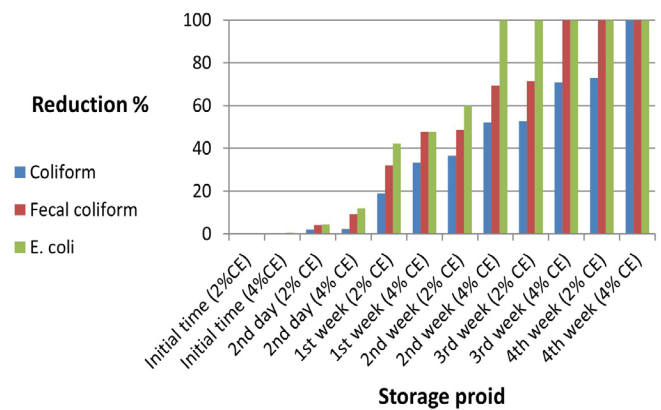


Figure 2. Reduction percentage (%) of coliform, fecal coliform and *E. coli* in butter samples after the addition of curcumin extract (CE).

The obtained data (Table 3 and Figure 3) showed that butter samples fortified with 2 and 4% curcumin extract reflected a 65.98 and 80.56% reduction in the psychrotrophic count, respectively at the end of storage. While, the lipolytic count in butter fortified with 2 and 4% curcumin extract exhibited a reduction of 72.76 and 82.91%, respectively by the fourth week.

The butter samples fortified with 2 and 4% curcumin extract had a 70.74 and 77.28% reduction in enterococci count, respectively. Statistically, the butter samples containing 2 and 4% curcumin extract showed significant differences ( $p < 0.05$ ) compared to the control sample in counts of psychrotrophic, lipolytic, and enterococci from the first to the last week of storage (Table 3 and Figure 3).

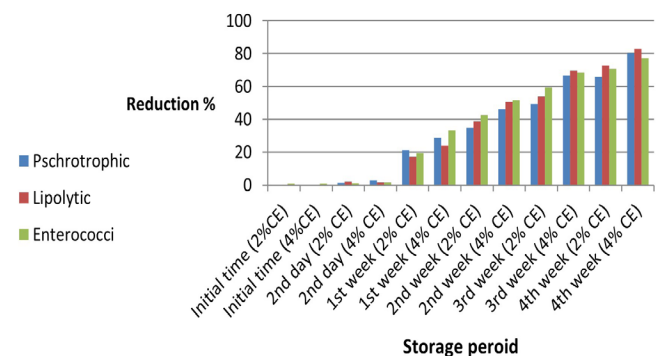


Figure 3. Reduction percentage (%) of psychrotrophic, lipolytic and enterococci in butter samples after the addition of curcumin extract (CE).

The results confirmed that the addition of curcumin extract to the butter samples significantly improved its microbial quality. These find-

ings are consistent with those of Betts *et al.* (2014), who mentioned that curcumin exhibits antimicrobial activity against a wide range of bacteria. The antimicrobial effects of curcumin are attributed to several mechanisms, including disruption of DNA replication, inhibition of plasmid gene expression, damage to cell membranes, and restriction of microbial movement (De *et al.*, 2009). Curcumin can be used as a broad-spectrum antibacterial agent either independently or in combination with specific antibiotics (Hussain *et al.*, 2022).

Table 4 and Figure 4 referred that butter samples with 2% curcumin extract demonstrated a 69.22 and 68.97% reduction in yeasts and molds, respectively. Additionally, the butter samples containing 4% curcumin extract having 100% reduction in both yeasts and molds by the fourth week of storage. Butter samples treated with 2 and 4% curcumin extract showed significant differences compared to the control in the case of yeasts and molds counts at the storage period range from the first to the fourth week as  $p < 0.05$ .

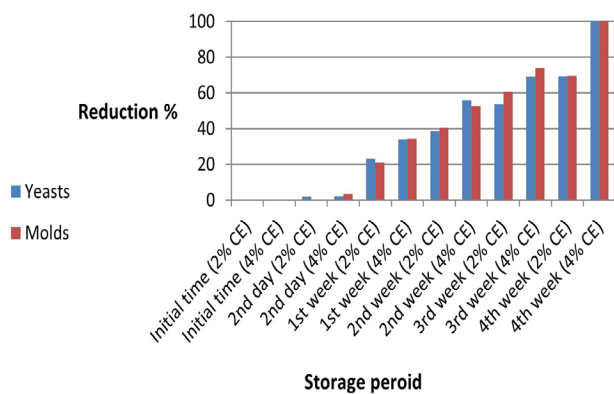


Figure 4. Reduction percentage (%) of yeasts and molds in butter samples after the addition of curcumin extract (CE).

These observations were matched with the result of Gul and Bakht (2015), who found that curcumin has a strong fungistatic effect. The antifungal properties of curcumin are likely associated with changes in ergosterol biosynthesis, the secretion of proteinases, and ATPase activity of the fungal cell membrane (Kim *et al.*, 2003).

## Conclusion

This finding cleared that cooking butter enriched with curcumin extract possesses desirable organoleptic characteristics, as well as antimicrobial, and antifungal properties when stored in the refrigerator. This research strongly suggests incorporating curcumin extract into butter as a natural preservative instead of artificial compounds without altering the sensory qualities.

## Conflict of interest

The authors have no conflict of interest to declare.

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