

Quality evaluation of Braided Cheese (*Gibna mudaffara*) made from raw milk with the addition of starter culture during the ripening period

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Abstract

The purpose of this study was to see what effect adding starter culture to raw milk had on the physicochemical, microbiological, and sensory properties of braided cheese (*Gibna mudaffara*). The raw milk was warmed to 45°C before adding 2% w/v starter culture to the first treatment (T1) and no starter culture to the second treatment (T2). Both treatments contained sodium chloride (2% w/w) and rennet powder (1.3 gm/50 L milk). After manufacturing, the cheese was kept in the whey at 4°C for 45 days. At 1, 15, 30, and 45-day intervals, physicochemical, microbiological, and sensory characteristics were assessed. Results revealed that protein (18.21%), total solids (50.27%), ash (7.29%), and titratable acidity (0.65%) were found to be high in T1 cheese, while fat (19.66%) and moisture (48.97%) were found to be high in T2. The ripening period had a significant ($P < 0.001$) effect on all physicochemical characteristics except fat. T2 cheese had high counts of total viable bacteria [TVB] (\log_{10} 6.60 cfu/g), *Staphylococcus aureus* (\log_{10} 2.35 cfu/g), *Escherichia coli* (\log_{10} 2.01 cfu/g), and yeasts and moulds (\log_{10} 5.91 cfu/g). In T1 cheese, all microbes under study decreased significantly as the ripening period progressed, whereas in T2 cheese, all microbes decreased during ripening except TVBC. T2 cheese scored higher on colour, flavour, and overall acceptability, while T1 cheese scored higher on taste and body. All sensory properties of both cheeses were significantly affected by the ripening period. This study concluded that heat treatment and adding the starter culture is required for the production of safe and high-quality braided cheese.

Keywords: Braided cheese; Raw milk; Quality; Ripening period

1. Introduction

Artisanal dairy products are those manufactured with traditional techniques using raw milk without heat treatment or the addition of selected starter cultures [1]. Cheese is a dairy product in which casein, fats, and other milk nutritional compounds remain in the curd, and the main goal of cheese making is to preserve the nutritional components in non-spoiling conditions without losing the desired flavour [2,3], and cheese comes in a wide variety of flavours, textures, and designs from around the globe [4]. In addition to other cheese types like mozzarella and white (*Gibna bayda*), which differ in composition, texture, colour, taste, and flavour, braided (*mudaffara*) cheese is thought to be one of the types made traditionally in Sudan [5]. *Mudaffara* cheese is a type of cheese that is braided and not aged, with a semi-hard texture. It originated in the Mediterranean region and is characterized by its dense consistency, yellowish colour, slightly acidic, and salty taste. This cheese is commonly consumed by rural communities in Sudan [6]. Many families and workers have historically consumed raw, untreated milk because they think it is safe and has health benefits that pasteurization destroys [7]. Many different types of bacteria can contaminate cheese made from raw milk, causing it to spoil or possibly endangering human health [8]. There are numerous factors that can influence the presence of spoilage and pathogenic microorganisms in raw milk, such as the condition of dairy animals, the hygienic milking process, storage conditions, farm management practices, location, and seasonal variations [9].

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As a common practice to inhibit microbial growth in milk, heat treatment has emerged as the most significant aspect of milk processing [10]. The complex microbial ecosystems that reside in milk and milk products are the primary cause of the wide range of tastes, aromas, and textures found in these foods. Pathogenic bacteria that contaminate milk and milk products are primarily brought on by handling, processing, and unsanitary conditions [11]. During the production and transportation of cheese, various sources such as mastitis milk, contaminated air, equipment used for storage and transportation, brine, starter cultures, floor and packaging materials, cheesecloth and curd cutting knives, cold room and production room air can all lead to microbial contamination of cheese [12,13]. Starter cultures are one or more strains of one or more desirable bacteria that are used to inoculate raw or pasteurized milk in order to produce fermented food by accelerating and guiding the fermentation process [14]. During cheese production, the main role of starter cultures is the production of lactic acid by metabolizing lactose, leading to the improvement of the milk coagulation process, making the curd stronger and protecting the final product against contamination [14].

The production of cheese necessitates the utilization of starter cultures comprising thermophilic/mesophilic lactic acid bacteria, which serve the primary purpose of generating lactic acid and flavoring compounds. Different microorganisms are employed in crafting various varieties of cheese, resulting in variations in taste, aroma, and texture [15]. The purpose of this study is to investigate how starter culture addition can affect the physicochemical, microbiological, and sensory aspects of braided cheese (*Gibna mudaffara*) made from raw milk.

2. Material and methods

Rennet powder and Direct Vat Set (DVS) starter culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*, 1:1 combination) were obtained from Chris Hansen's Laboratory (Denmark). The salt was purchased from a local market.

2.1. Cheese manufacture

The cheese was produced according to the method of Harun et al. [16]. Fourteen liters (14 L) of fresh whole cow's milk was heated to 45°C, followed by addition of sodium chloride (2% w/w). The milk was then transferred to two stainless steel containers (7 L each) and subsequently cooled to 42°C. The starter culture (2% w/v) was added to the first treatment [T1], while no starter culture was added to the second treatment [T2]. The milk was left undisturbed for 30 minutes to develop acidity, then rennet powder (1.3 gm/50 L milk) was dissolved in 50 ml distilled water and added to both treatments at 40°C. The milk was then stirred thoroughly and allowed to stand to form a curd, which was then cut with a sterile knife to drain the whey and placed in an incubator (40°C) for 3 hours until the curd was ready to boil in water (75°C) for 5 minutes to promote sufficient elasticity. The curd from both treatments was formed into balls and stretched on the clean table, the black cumin (0.5% w/w) was mixed with the curd and braided into the final shape, and the braided curd was kept in salted whey (10% w/w) for 24 hours, after which the cheese was kept in the whey at 4°C for 28 days. The physicochemical, microbiological and sensory characteristics were determined at intervals of 1, 15, 30 and 45 days. The production of the cheese was carried out in triplicate.

2.2. Physicochemical analyses of milk

The physicochemical analyses were carried out on a sample of mixed whole raw milk. The fat, total solids, protein contents and titratable acidity were determined according to the standard methods of the Association of Official Analytical Chemists [17].

2.3. Physicochemical analysis of cheese

The fat, protein, total solids, ash contents, and acidity were determined according to AOAC [17], while the moisture content was determined by subtraction (100% - total solids%).

2.4. Microbiological examination of cheese

2.4.1. Preparation of samples

The sample (11 g) was aseptically weighed into a sterile blender container and 99 ml of sterile peptone water was added and mixed for 2 minutes to make a 10^{-1} dilution. A tenfold dilution was made with a sterile peptone water up to 10^{-8} . After inoculation at the required temperature, the results were calculated as colony forming units per gram of sample (cfu/g) [18].

2.4.2. Total viable bacterial count (TVBC)

Plate count agar medium (Himedia, M091) was used for the determination of TVB. The plates were incubated in an inverted position at 37°C for 48 hours. The colonies of each plate were counted using a manual colony counter (Scan 100) and the total number of viable bacteria was calculated as cfu/g [19].

2.4.3. *Staphylococcus aureus* count

Mannitol salt agar (Micro master, DM160) was used for the enumeration of coagulase-positive staphylococci. Plates were incubated for 48 hours in an inverted position at 37°C and colonies were counted using a manual colony counter (Scan 100) and considered as cfu/g [18]. Suspected colonies of *S. aureus* were confirmed by Gram stain and various biochemical tests, including catalase test, oxidase test, indole production, methyl red, Voges-Proskauer test, nitrate reduction, acid from various sugars and coagulase test [20].

2.4.4. *Escherichia coli* count

Brilliant Green Lactose Bile (BGB) broth (Merck, 736) and peptone water (Himedia, M028) were used for the enumeration of *E. coli* using the most probable number technique. Eosin methylene blue agar (EMB) (Millipore, 70186) was used to confirm the presence of *E. coli* [18]. Four to five suspicious colonies were taken from each plate, cultured and identified by various biochemical tests: Gram stain, catalase test, indole, methyl red, Voges-Proskauer test, nitrate reduction, urease production, Simon citrate agar and various sugar fermentation tests [20].

2.4.5. Yeasts and moulds count

Yeast extract agar medium (Himedia, M456) was used for the enumeration of yeasts and moulds. Plates were incubated in an inverted position at 25°C for 5 days and colonies were counted using a manual colony counter (Scan 100) and recorded as cfu/g [21].

2.5. Sensory evaluation of cheese

The samples were left to stand at room temperature for two hours before sensory testing. All samples were presented in plastic trays. A panel of ten untrained tasters aged 25-30 (6 women and 4 men) who were familiar with the product were selected and asked to assess the quality of the cheese (colour, flavour, body, taste, saltiness and overall acceptability) using a score sheet, with colour ranging from 1= unacceptable to 4 = acceptable; flavour 1 = bland to 4 = extremely intense; taste 1 = non-existent to 4 = too sour; body 1 = soft to 4 = pasty; saltiness 1 = moderate to 4 = too salty; overall acceptability 1 = unacceptable to 4 = acceptable [22].

2.6. Statistical analysis

Statistical analyses were performed using the Statistical Analysis Systems (SAS, ver. 9). A factorial design (2×4) was used to determine the influence of starter culture and ripening period on the physicochemical, microbiological and sensory properties of the braided cheese (*Gibna mudaffara*). Duncan's Multiple Range Tests were performed for mean separation between treatments ($P \leq 0.05$).

3. Results and discussion

The physicochemical characteristics of the raw milk from which the cheese was made are 5.2%, 3.62%, 13%, 0.7% and 0.2% for fat, protein, total solids, ash content and titratable acidity, respectively.

3.1. Effect of starter culture on the physicochemical properties of cheese

The physicochemical properties of cheese produced with and without starter culture are shown in Table 1. All physicochemical properties were not significantly affected by the addition of starter cultures ($P > 0.05$), except ash content and titratable acidity. The highest protein, total solids, ash contents and titratable acidity were reported in cheese made with starter culture (T1), while the highest fat and moisture contents were found in cheese made without starter culture (T2). The starter culture addition increased the contents of protein, total solids, ash contents and titratable acidity, and did not affect the contents of moisture and fat. The chemical composition and physical properties of cheese are influenced by many factors, such as the type of milk, the season of milk production, the fat separation method used, the microflora of the milk and/or the addition of starter cultures, the additives in the milk and the storage time and temperature [23]. Increasing the rate of starter culture in heat-treated buffalo milk Feta type cheese was found to increase fat, protein, moisture content, and decrease total solids content [24]. According to Vapur et al. [15], white cheese made with 80% thermophilic cultures plus 20% mesophilic cultures had a slightly higher fat content and pH

than cheese made with 60% thermophilic cultures plus 40% mesophilic cultures, and this increase was attributed to the thermophilic culture's activity. The results of this study are consistent with the findings of Salih and Abdalla [5], who found that the addition of starter cultures had no significant effect ($P>0.05$) on all physicochemical properties of white cheese made from pasteurized milk with and without starter culture addition, with the exception of ash content, which was higher in cheese made with the addition of starter cultures. The protein content result is contrary to Hussein and Shalaby [25], who reported that cheese made with yoghurt starter showed an increase in total protein content, and to Najafi et al. [26], who reported that the protein content of cheese increased with the addition of starter cultures. The total solids result agrees with that of Sert et al. [27], who reported that the use of culture leads to a higher titratable acidity in the cheese, which increases the extent of whey separation and leads to an increase in total solids. The moisture content result is in agreement with Ekici et al. [28], who found that the starter culture did not significantly affect the moisture content. When more starter cultures were added to buffalo milk feta type cheese, its fat content, fat content in dry matter, protein content, and salt content increased. However, when more starter cultures were added to the cheese-making process, its total solids content decreased [24]. Mudawi et al. [29] reported that increasing the concentration of starter culture added to milk for the production of white cheese from 0% to 2.5% increased the moisture content and acidity, and decreased the fat, protein and total solids content. In their analysis of costeño cheese, Fajardo et al. [30] found that the use of starter culture increased the acidity of the cheese, while pasteurized milk without starter culture produced cheese with lower acidity. The relationship between acidity and lactic acid production from lactic acid bacteria may account for the higher acidity of cheese made with starter culture addition as compared to cheese made without it [30].

Table 1 Effect of starter culture addition on the physicochemical characteristics of cheese made from raw milk

Physicochemical characteristics (%)	Treatment		SE	SL
	T1	T2		
Fat	19.28 ^a	19.66 ^a	0.544	NS
Protein	18.21 ^a	17.77 ^a	0.662	NS
Total solids	50.27 ^a	50.23 ^a	0.819	NS
Moisture	49.73 ^a	48.97 ^a	0.821	NS
Ash	7.29 ^a	6.51 ^b	0.260	**
Acidity	0.65 ^a	0.48 ^b	0.041	**

Means in each row bearing similar superscripts are not significantly different ($P>0.05$); NS = Not significant; SL = Significance level; SE = Standard error of means; T1 = cheese made with starter culture addition; T2 = Cheese made without starter culture addition

3.2. The physicochemical properties of cheese during the ripening period

The results in Table 2 show the effect of ripening period on the physicochemical properties of raw milk cheese produced with and without a starter culture. The ripening period significantly affected all physicochemical properties, except fat content in both treatments, which fluctuated during the ripening period reaching the lowest (18.97%) and highest (20.09%) contents on days 15 and 45, respectively in T1 cheese, while T2 cheese showed an insignificantly ($P>0.05$) increasing trend from 18.75% on day1 to 20.00% on day 45. This result disagrees with the that reported by Salih and Abdalla [5] who reported an increasing trend in fat content during the storage period of white cheese made from pasteurized milk with the addition of starter cultures, and Kheir et al. [31] who reported an increasing trend in fat content of white cheese made from heated milk without the addition of starter cultures. The protein content significantly ($P<0.001$) decreased in both treatments during the ripening period, from 20.16% on day 1 to 15.91% on day 45 in T1 cheese, and 20.90% on day 1 to 15.20% at the end of the ripening period.

This result agrees with Harun et al. [16] who found a decreasing trend of protein content in *mudaffara* cheese from $22.30\pm 1.47\%$ at the beginning of the storage period to $21.61\pm 3.57\%$ at the end. Salih and Abdalla [5] concluded that the protein content of white cheese made from pasteurized milk with and without starter culture increased significantly during the storage period. However, the results contradict the findings of Kheir et al. [31], who found an increasing trend in the protein content of white cheese from pasteurized milk produced with both rennet and *Solanum dubium* as coagulants without the addition of starter cultures. The decrease in protein content during storage could be due to protein degradation, which leads to the formation of water-soluble compounds [32]. Total solids significantly ($P<0.01$) decreased from 50.53% (T1) and 52.95% (T2) on day 1 to 48.83% (T1) and 49.70% (T2) on day 30 before slightly increasing to 49.20% (T1) and 49.96% (T2) at the end of the ripening period. The result in this study is in disagreement

with Harun et al. [16] who reported an increasing trend of total solids content in *mudaffara* cheese from $45.102 \pm 3.73\%$ at the beginning to $58.45 \pm 15.70\%$ at the end of the storage period of 28 days. In a previous study, an increase in the total solids content of white cheese made from pasteurized milk with and without added starter cultures was observed during the 45-day storage period [5].

Table 2 Physicochemical characteristics of raw milk cheese made with and without starter culture during the ripening period

Physicochemical characteristics (%)	Ripening Period (days)				SE	SL
	1	15	30	45		
With starter [T1]						
Fat	19.75 ^a	18.97 ^a	19.47 ^a	20.09 ^a	0.192	NS
Protein	20.16 ^a	18.18 ^b	17.06 ^b	15.91 ^c	0.234	***
Total solids	50.53 ^a	49.46 ^a	48.83 ^b	49.20 ^a	0.289	**
Moisture	49.48 ^a	49.04 ^a	48.12 ^b	49.80 ^a	0.290	*
Ash	8.00 ^a	7.66 ^b	6.71 ^b	6.70 ^b	0.092	***
Acidity	0.52 ^b	0.57 ^b	0.75 ^a	0.77 ^a	0.015	***
Without starter [T2]						
Fat	18.75 ^a	19.94 ^a	19.98 ^a	20.00 ^a	0.784	NS
Protein	20.90 ^a	19.29 ^b	16.49 ^c	15.20 ^d	0.656	***
Total solids	52.95 ^a	50.70 ^b	49.70 ^c	49.96 ^b	1.040	**
Moisture	48.05 ^b	49.30 ^a	49.00 ^a	49.54 ^a	1.040	**
Ash	8.92 ^a	5.66 ^b	5.84 ^b	5.63 ^b	0.336	***
Acidity	0.21 ^c	0.50 ^a	0.65 ^b	0.51 ^a	0.063	***

Means in each row bearing similar superscripts are not significantly different ($P > 0.05$); *** = $P < 0.001$; NS = Not significant; SL = Significance level; SE = Standard error of means

The moisture content significantly increased during the ripening period reaching the maximum content of 49.80% at the end of the ripening period in T1 and 49.54% in T2. These results agree with those of El Siddig et al. [33], who reported an increasing trend in the moisture content of white cheese during the storage period of four months, and disagree with those of Salih and Abdalla [5], who reported that the moisture content of pasteurized milk white cheese produced with and without starter culture decreased during the storage period. Ash content significantly ($P < 0.001$) decreased in both treatments from 8.00% (T1) and 8.92% (T2) on day 1 to 6.70% (T1) and 5.63% (T2) on day 45 of the ripening period. The results agree with Salih and Abdalla [5] for white cheese, and disagree with Harun et al. [16] who reported an increasing trend in ash content of *mudaffara* cheese from $5.53 \pm 0.51\%$ at the beginning of the storage period to $17.18 \pm 12.98\%$ at the end. The titratable acidity significantly ($P < 0.001$) increased with ripening period in both treatments from 0.52% (T1) and 0.21% (T2) at the beginning of the ripening period to 0.77% (T1) and 0.51% (T2) at the end of the ripening period. This result is consistent with that of Harun et al. [16] who reported an increasing trend of the acidity of *mudaffara* cheese from $0.42 \pm 0.11\%$ on day 1 to $0.50 \pm 0.09\%$ on day 28 of the storage period. The increase in acidity during the ripening period may be due to the formation of acidity through the activity of cheese lactic acid bacteria [5,33].

3.3. Effect of starter culture on the microbiological characteristics of cheese

The microbiological quality (\log_{10} cfu/g) of the cheese, which was influenced by the addition of starter cultures, is shown in Table 3. All microbiological properties were significantly higher in cheese made without addition of starter culture (T2). Total viable bacteria (TVB) (\log_{10} 6.60 cfu/g), *S. aureus* (\log_{10} 2.35 cfu/g), *E. coli* (\log_{10} 2.01 cfu/g) and yeasts and moulds (\log_{10} 5.91 cfu/g) counts were higher in T2, compared to counts in cheese made with starter culture (T1). These results agree with those of Salih and Abdalla [5], who

Table 3 Effect of starter culture addition on the microbiological characteristics (\log_{10} cfu/g) of cheese made from raw milk

Microbiological characteristics	Treatment		SE	SL
	T1	T2		
TVB	5.54 ^b	6.60 ^a	0.015	**
<i>S. aureus</i>	1.58 ^b	2.35 ^a	0.162	**
<i>E. coli</i>	0.26 ^b	2.01 ^a	0.382	***
Yeasts and moulds	4.53 ^b	5.91 ^a	0.037	***

Means in each row bearing similar superscripts are not significantly different ($P>0.05$); *** = $P<0.001$; NS = Not significant; SL = Significance level; SE = Standard error of means

found that the addition of the starter affected the microbiological quality of the cheese, with the total number of viable bacteria, *S. aureus* and yeasts and moulds being significantly ($P<0.05$) lower in cheese with the addition of the starter culture. The result of TVBC contradicts the results of Mudawi et al. [29], who showed that the total bacterial count was highest (4.1×10^6 cfu/g) in cheese made with a 2.5% starter culture and lowest (2.3×10^4 cfu/g) in cheese made without a starter culture. *S. aureus* count result is consistent with Arques et al. [30], who found that *S. aureus* counts were lower in cheese made with a starter culture than in cheese made without a starter culture. White cheese made with 80% thermophilic culture plus 20% mesophilic culture and cheese made with 60% thermophilic culture plus 40% mesophilic culture did not differ significantly ($P>0.05$) in the *S. aureus* count, according to Vapur et al. [15]. The decrease in the number of *S. aureus*, as well as *E. coli* can be attributed to the starter cultures' inhibitory effect against pathogenic and spoilage microorganisms, which includes the production of organic acids and subsequent pH reduction, acting as strong competitors for nutritional factors like nicotinamide, biotin, or nicin [35,36,37]. The result of the yeast and mould count agrees with that of Salih and Abdalla [5] who found that yeasts and moulds count was significantly ($P<0.001$) higher in white cheese made without starter culture compared to that made with starter culture, and Shah [38], who reported that lactic acid bacteria produce some components that have a bactericidal and bacteriostatic effect, leading to the retardation and/or disappearance of fungal growth in the samples during the storage period. The yeast count was significantly ($P<0.01$) higher in white cheese made with 80% thermophilic culture plus 20% mesophilic culture compared to cheese made with 60% thermophilic culture plus 40% mesophilic culture [15].

3.4. The microbiological properties of cheese during the ripening period

The results in Table 4 show that the ripening period significantly affected the microbiological properties of both treatments under study. For cheese made with starter culture (T1), all microbes steadily decreased towards the end of the ripening period, except TVBC which decreased to the lowest count of \log_{10} 5.40 cfu/g on day 30 before increasing again towards the end. For cheese made without starter culture (T2), all microbes under study increased during the ripening period, except TVBC which decreased as the ripening period progressed. The results of TVBC agree with those of Al-Ghamdi et al. [39], who found decrease in the total plate count of white cheese during the 60-day storage period, and Abdalla and Omer [40], who described that the total plate count increased from \log_{10} 7.68 cfu/g in raw milk to \log_{10} 7.91 cfu/g in milk delivered to the market when white cheese was processed using traditional methods. Abdalla and Mohammed [41] described that the total viable bacteria count increased insignificantly from \log_{10} 8.10 ± 0.047 cfu/g on day 1 to \log_{10} 8.20 ± 0.047 cfu/g on day 60. The number of *S. aureus* decreased significantly ($P<0.001$) during ripening from \log_{10} 2.62 cfu/g on day 1 to \log_{10} 0.95 cfu/g on day 45 in T1 cheese, and increased from \log_{10} 2.29 cfu/g on day 1 to \log_{10} 2.87 cfu/g on day 45 in T2 cheese. The decrease in *S. aureus* count in T1 cheese may be due to the effect of acid produced by lactic acid bacteria which lead to decreasing the number of bacteria [40], while the increase in the count in T2 cheese was mainly due to the lack of the effect of starter culture. Abdalla and Omer (2017) found a decreasing trend of *S. aureus* during the processing of white cheese using traditional methods, and Al-Ghamdi et al. [39] reported an insignificant increase in *S. aureus* during the 60-day storage period of white cheese. Kheir et al. [31] reported that *S. aureus* fluctuated during the storage period of white cheese made with rennet without starter culture, reaching the maximum count of 9.02×10^4 cfu/g on day 60. *E. coli* decreased on the 15th day of ripening and not detected thereafter in T1 cheese, while in T2 cheese significantly ($P<0.001$) increased from \log_{10} 1.50 cfu/g on day 1 to \log_{10} 2.58 cfu/g on day 45. In a previous study it was reported that the use of lactic acid bacteria in Pasta Filata cheese manufacture resulted in a decrease in the total number of *E. coli* count compared to the control cheese [42].

Table 4 Microbiological characteristics (\log_{10} cfu/g) of raw milk cheese made with and without starter during the ripening period

Microbiological characteristics	Ripening Period (days)				SE	SL
	1	15	30	45		
With starter [T1]						
TVB	7.08 ^a	5.95 ^b	5.40 ^b	4.59 ^c	0.024	***
<i>S. aureus</i>	2.62 ^a	1.37 ^b	1.32 ^a	0.95 ^a	0.057	***
<i>E. coli</i>	0.72 ^a	0.32 ^b	ND	ND	0.093	*
Yeasts and moulds	5.31 ^a	4.92 ^a	4.15 ^b	4.09 ^b	0.032	***
Without starter [T2]						
TVBC	7.88 ^a	6.31 ^b	6.32 ^b	5.89 ^c	0.100	***
<i>S. aureus</i>	2.29 ^c	1.75 ^d	2.62 ^b	2.87 ^a	0.228	***
<i>E. coli</i>	1.50 ^c	1.97 ^b	2.00 ^b	2.58 ^a	0.128	**
Yeasts and moulds	5.15 ^b	5.64 ^b	6.44 ^a	6.58 ^a	0.139	*

Means in each row bearing similar superscripts are not significantly different ($P>0.05$); *** = $P<0.001$; NS = Not significant; SL = Significance level; SE = Standard error of means

The results in T1 cheese agree with the findings of ElOwani and Hamid [43], who reported that the number of *E. coli* decreased during the storage period of white cheese from \log_{10} 2.14 \pm 0.16 cfu/g on day 0 to \log_{10} 0.16 \pm 0.43 cfu/g on day 120 and was not detected thereafter. Salih and Abdalla [5] reported that *E. coli* was not detected on days 1 and 45 of the storage period of white cheese made from pasteurized milk without a starter culture and on days 1 and 30 for cheese made with a starter culture. The number of yeasts and moulds decreased from \log_{10} 5.31 cfu/g on day 1 to \log_{10} 4.09 cfu/g on day 45 in T1 cheese, while in T2 cheese the number increased from \log_{10} 5.15 cfu/g at the beginning to \log_{10} 6.58 cfu/g at the end of the ripening period. The results of T2 cheese agree with the findings of Salih and Abdalla [5] in white cheese made without starter culture, while the results of T1 cheese disagree with their findings. Previous studies reported that yeasts and moulds increased during the storage period of white cheese [33,39].

3.5. Effect of the starter culture on the sensory properties of cheese

The sensory properties of the cheese, which were influenced by the addition of starter cultures, are shown in Table 5. The addition of starter culture significantly affected all sensory properties except the colour.

Table 5 Effect of starter culture addition on the sensory characteristics of cheese made from raw milk

Sensory characteristics	Treatment		SE	SL
	T1	T2		
Colour	3.16 ^a	3.30 ^a	0.037	NS
Flavour	2.53 ^b	2.70 ^a	0.039	*
Taste	2.43 ^b	2.08 ^a	0.031	***
Body	2.26 ^a	1.85 ^b	0.030	***
Saltiness	1.44 ^a	1.28 ^b	0.019	**
Overall acceptability	2.79 ^a	3.20 ^b	0.046	***

Means in each row bearing similar superscripts are not significantly different ($P>0.05$); *** = $P<0.001$; NS = Not significant; SL = Significance level; SE = Standard error of means

The taste (2.43) and body (2.26) of cheese made with starter culture (T1) scored better, and cheese was slightly salty than cheese made without starter culture (T2), while the colour (3.30), flavour (2.70) and overall acceptability (3.20) scored better in cheese made without starter culture (T2). A previous study found no significant differences ($P>0.05$)

in colour, appearance, odour, taste, flavour, and overall acceptability between white cheese made with 80% thermophilic culture plus 20% mesophilic culture and white cheese made with 60% thermophilic culture plus 40% mesophilic culture, but there was a significant difference ($P < 0.05$) in body and texture [15]. The overall acceptability result agrees with Frau et al. [44], who found that cheese made with commercial starters had lower acceptability due to its strong acidic taste. The colour and flavour results are in disagreement with Sabbagh et al. [45], who reported that the addition of adjunct cultures had a significant effect on flavour and colour. The result of body is in agreement with Sulejmani et al. [46] and Kourkoutas et al. [47], who found that Feta cheese made with freeze-dried cultures had better texture and structure, probably due to the effect of lactic acid bacteria and the formation of slightly acidic conditions that facilitate the action of the rennet. The salt content was not significantly affected by the addition of starter cultures. Mudawi et al. [29] reported that as the concentration of starter culture used for cheese making increased, the colour deteriorated and reached the lowest score for cheese made with 2.5% starter culture, while the best scores for taste, texture and acceptability were obtained for cheese made with 1.5% starter culture. Salih and Abdalla [5] found that the addition of starter cultures in the production of white cheese improved the colour, flavour and overall acceptability, while the body of the cheese deteriorated.

3.6. The sensory properties of cheese during the ripening period

Table 6 shows that the ripening period significantly affected all sensory properties, and cheese of both treatments scored better in all sensory properties during the ripening period, and cheese became salty, indicating that the addition of starter culture resulted in better sensory characteristics and increased the ripening index. These results are consistent with Mohammed and Abdalla [48] who reported that the colour of cooked and unheated white cheese improved until the 40th day of storage and then decreased. Salih and Abdalla [5] stated that during the ripening period, the taste, flavour, texture and overall acceptability of white cheese made with and without starter culture improved, while the colour deteriorated. The improvement in flavour may be due to the formation of lactic acid or from the natural flora of the raw milk or from the yeast culture added to the milk before cheese making, resulting in the suppression of unwanted microorganisms [43].

Table 6 Sensory characteristics of raw milk cheese made with and without starter Culture during the ripening period

Sensory characteristics	Ripening Period (days)				SE	SL
	1	15	30	45		
With starter [T1]						
Colour	3.23 ^{ab}	3.19 ^{ab}	3.41 ^a	3.09 ^b	0.009	**
Flavour	2.45 ^b	2.38 ^b	2.70 ^a	2.93 ^a	0.009	***
Taste	1.90 ^c	2.31 ^b	2.20 ^b	2.59 ^a	0.008	***
Body	1.43 ^b	2.30 ^a	2.19 ^a	2.31 ^a	0.010	***
Saltiness	1.10 ^d	1.65 ^a	1.26 ^c	1.43 ^b	0.006	***
Overall acceptability	2.83 ^b	3.15 ^a	3.08 ^{ab}	2.94 ^{ab}	0.009	*
Without starter [T2]						
Colour	3.00 ^b	2.90 ^b	3.75 ^a	3.55 ^a	0.139	***
Flavour	2.80 ^a	2.40 ^b	2.75 ^{ab}	2.85 ^a	0.127	*
Taste	2.00 ^b	2.45 ^a	1.65 ^c	2.20 ^{ab}	0.120	***
Body	1.30 ^c	2.35 ^a	1.80 ^b	1.95 ^b	0.103	***
Saltiness	1.00 ^c	1.70 ^a	1.10 ^{bc}	1.30 ^b	0.076	***
Overall acceptability	2.90 ^b	3.15 ^{ab}	3.40 ^a	3.35 ^a	0.124	*

Means in each row bearing similar superscripts are not significantly different ($P > 0.05$); *** = $P < 0.001$; NS = Not significant; SL = Significance level; SE = Standard error of means

4. Conclusion

In the cheese making process, adding starter culture to milk is an important step in improving the cheese during the processing and subsequent ripening. This represents an improvement in the cheese's microbiological quality, as well as an improvement in the cheese's physicochemical and sensory characteristics. The physicochemical properties improved as well as its quality and sensory properties. During ripening, the cheese's microbiological quality improved as well. Pasteurization of milk and the addition of starter culture for the manufacture of *mudaffara* cheese should therefore be encouraged in order to improve the quality of *mudaffara* cheese and ensure that the use of starter culture in the production of handmade white cheese is a safe product.

Compliance with ethical standards

Disclosure of conflict of interest

The author declares that there is no conflict of interest.

References

- [1] Gebril EA, Abo El-Makarem HS, Amer AA. (2021). Microbial safety of artisanal dairy products in comparison with Egyptian specifications. *Alex J Vet Sci.* 2021; 69 (1): 61-69.
- [2] Béjar-Lio GI, Gutiérrez-Méndez N, Nevárez-Moorillón GV. Microbiological and physicochemical characteristics of Chihuahua cheese manufactured with raw milk. *AIMS Agric Food.* 2020; 5(1): 86–101.
- [3] Al-Ghamdi AY, Salih HAM, Abdalla MOM. Microbiological, physicochemical, and sensory characteristics of traditional white soft cheese (Gibna bayda) supplemented with commercial starter culture. *GSC Adv Res Rev.* 2021; 7(2): 6-15.
- [4] Umayra AN, Hassan JS, Abdul-Hadi HF. Isolation and identification of Gram-positive and Gram-negative bacteria from different types of cheese in Baghdad's local markets. *Biomed Chem Sci.* 2023; 2(3): 186 – 189.
- [5] Salih HAM, Abdalla MOM. Effect of starter addition on the physicochemical, microbiological and sensory characteristics of pasteurized milk white cheese (Gibna bayda). *Asian Food Sci J.* 2020; 15(4): 32-44, 2020; Article no. AFSJ. 56785.
- [6] Abdalla MOM, Gamer Eldin HEA. Effect of milk type and salt concentration on the physicochemical characteristics of mudaffara (braided) cheese. *Adv Res.* 2017; 13(5): 1-9, 2018; Article no. AIR.33121.
- [7] Megersa R, Mathewos M, Fesseha H. Isolation and identification of *Escherichia coli* from dairy cow raw milk in Bishoftu Town, Central Ethiopia. *Arch Vet Anim Sci.* 2019; 1(1).
- [8] Imre K, Ban-Cucerzan A, Herman V, Sallam KI, Cristina RT, Abd-Elghany SM, Morar D, Popa SA, Imre M, Mora A. Occurrence, pathogenic potential and antimicrobial resistance of *Escherichia coli* isolated from raw milk cheese commercialized in Banat Region, Romania. *Antibiot.* 2022; 11: 721. <https://doi.org/10.3390/antibiotics11060721>.
- [9] Al-Shuwaili AM, Khudhir SZ. Evaluation of the bacterial load in the raw dairy products in Baghdad, Iraq. *Arch Razi Instit.* 2022; 77(6): 2319-2328.
- [10] Abdalla MOM, Salih HAM. Effect of heat treatment of milk on the physicochemical, microbiological, and sensory characteristics of white cheese (Gibna bayda). *GSC Adv Res Rev.* 2020; 03(03): 020–028.
- [11] Kabeta T, Abera S, Kebede G, Sanbata D, Duguma A. Isolation and identification of *Staphylococcus* species from cottage cheese and yoghurt in selected districts of East Wollega Zone, Ethiopia. *Austin J Vet Sci Anim Husband.* 2023; 10(1): 1114.
- [12] Zeinoh, M.M.A., & Abed, A.H. (2021). Prevalence, characterization, and control of *Staphylococcus aureus* isolated from raw milk and Egyptian soft cheese. *J Vet Med Res.*, 27 (2), 152–160.
- [13] Zain El-din, A.H., Abd El-Rahim, A.M., El-Gazzar, F.E., Osman, D.M., & Mahmoud, G.A. (2022). Microflora of some cheese types in Assiut City, Egypt. *Assiut J Agric Sci.*, 53(5), 13-23.
- [14] Bezie A, Regasa H. The role of starter culture and enzymes/rennet for fermented dairy products manufacture- A Review. *Nutr. Food Sci Inter J.* 2019; 9(2): 555756.

- [15] Vapur UE, Ersan LY, Özcan T. Effects of Starter Culture Combination on the Characteristic of White Cheese. *Int J Innov Approaches Agric Res.* 2019; 3 (2): 277-286.
- [16] Harun KII, Abdalla MOM, ElOwni OAO. Chemical composition of Mudaffara cheese manufactured by *Solanum dubium* coat extract and chymosin. *UK J Vet Med Anim Prod.* 2015; 6(1): 27-37.
- [17] AOAC. Official methods of analysis of AOAC international (17th edition). Official Methods. 2000; 920.124, 926.08, 955.30, 2001.14. Gaithersburg, MD: AOAC International.
- [18] Harrigan WF. *Laboratory Methods in Food Microbiology*, 3rd ed. 1998; Academic Press Ltd, London, England.
- [19] Houghtby AG, Maturin LJ, Koenig KE. (1992). *Microbiological Count Methods*. In: Marshal RT (Ed.), *Standard Methods for the Examination of Dairy Products*, 16th Ed. American Public Health Association, Washington, DC, USA. 1992; pp. 213-246.
- [20] Singh P, Prakash A. (2008). Isolation of *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes* from milk products sold under market conditions at Agra Region. *Acta Agr Slov.* 2008; 92: 83–88.
- [21] Frank FJ, Christen GL, Bullerman LB. Tests for groups of microorganisms. In: Marshal RT (Ed.), *Standard Methods for the Examination of Dairy Products*, 16th Ed. American Public Health Association, Washington, DC, USA. 1992; pp. 271-286.
- [22] Lim J. Hedonic scaling: A review of methods and theory. *Food Qual Prefer.* 2011; 22: 733-747.
- [23] Awad S. Microbial safety criteria and quality of traditional Egyptian Karish cheese. *Afr J Microbiol Res.* 2016; 10(22): 804-812.
- [24] Kumar SS, Kanawjia K, Kumar S, Khatkar S. (2014). Effect of rate of addition of starter culture on textural characteristics of buffalo milk Feta type cheese during ripening. *Food Sci Technol.* 2014; 51(4): 800-804.
- [25] Hussein MAG, Shalaby MS. (2014). Microstructure and textural properties of Kareish cheese manufactured by various ways. *Annals Agric Sci.* 2014; 59(1): 25–31.
- [26] Najafi NM, Koocheki A, Mahdizadeh M. Studies on the effect of starter culture concentration and renneting pH on the Iranian brine cheese yield. *Amer-Eurasian J Agri Environ Sci.* 2008; 3(3): 325-332.
- [27] Sert D, Ayar A, Akin N. The effects of starter culture on chemical composition, microbiological and sensory characteristics of Turkish Kasar cheese during ripening. *Internet J Food Safety.* 2007; 9: 7-13.
- [28] Ekici K, Tarakci Z, Alemdar S, Alisarli M. (2010). Influence of starter cultures on the accumulation of histamine and tyramine in vacuum-packaged Otlu (Herby) cheese during ripening. *Asian J Chem.* 2010; 22: 6133-6139.
- [29] Mudawi HA, Khairalla LM, El Tinay AH. Evaluation of the effect of starter culture on the quality of white soft cheese (Gibna beyda). *U K J Vet Med Anim Prod.* 2016; 7(2): 104-110.
- [30] Fajardo JGS, Ossa TIP, Hernández EJR. Effect of pasteurization and starter cultures on physicochemical and microbiological properties of costeño cheese. *Revista Facultad Nacional de Agronomía-Medellín.* 2016; 69(2): 8007-8014.
- [31] Kheir SEO, ElOwni OAO, Abdalla MOM. Comparison of quality of Sudanese white cheese (Gibna bayda) manufactured with *Solanum dubium* fruit extract and rennet. *Pak J Nutr.* 2011; 10 (2): 106-111.
- [32] Hamid AI. Effect of cumin oil concentrations on chemical composition and sensory characteristics of Sudanese white cheese during ripening. *Int. J. Curr. Microbiol. Appl. Sci.* 2014; 3(4): 961-968.
- [33] El-Siddig EE, Abdelgadir WS, Kabeir BM, Koko MYF, Ibrahim RA. Quality of white cheese made using *Moringa oleifera* leaf extract. *J Acad Indu. Res.* 2018; 7(1): 7-17
- [34] Arqués JL, Rodríguez E, Gaya P, Medina M, Guamis B, Nuñez M. Inactivation of *Staphylococcus aureus* in raw milk cheese by combinations of high-pressure treatments and bacteriocin-producing lactic acid bacteria. *J Appl Microbiol.* 2005; 98(2): 254-260.
- [35] Makrasa L, Triantafyllouab V, Fayol-Messaoudic F, Asianya T, Zoumpopouloub Z, Tsakalidoub E, Servinc A, Vuyst L. Kinetic analysis of the antibacterial activity of probiotic lactobacilli towards *Salmonella enterica* serovar Typhimurium reveals a role for lactic acid and other inhibitory compounds. *Res Microbiol.* 2006; 157(3): 241-247.
- [36] DeVuyst L, Leroy F. (2007). Bacteriocins from lactic acid bacteria: production, purification and food applications. *J. Molec. Microbiol. Biotechnol.* 2007; 13(4): 194-199.

- [37] Charlier C, Cretenet M, Even S, LeLoir Y. Interactions between *Staphylococcus aureus* and lactic acid bacteria: An old story with new perspectives. *Int J Food Microbiol.* 2009; 131: 30-39.
- [38] Shah NP. Functional cultures and health benefits. *Int Dairy J.* 2007; 17: 1262-1277.
- [39] Al-Ghamdi AY, AbdelKareem SAK, AlSharqi LEO, Abdalla MOM. Effect of preservation method on the microbiological characteristics of white cheese (Gibna bayda) during the storage period. *J Chem Biol Phys Sci.* 2020; 10(3): 368-378.
- [40] Abdalla MOM, Omer HEA. Microbiological characteristics of white cheese (Gibna bayda) manufactured under traditional conditions. *J Adv Microbiol.* 2017; 2(3): 1-7, 2017; Article no. JAMB.33152.
- [41] Abdalla MOM, Mohammed EHS. Effect of storage period on the microbiological and sensory characteristics of cooked low salt white soft cheese (Gebna Beyda). *Pak J Nutr.* 2010; 9 (3): 205-208.
- [42] Medved'ová A, Koňuchová M, Kvočiková K, Hatalová I, Valík L. Effect of lactic acid bacteria addition on the microbiological safety of Pasta-Filata types of cheeses. *Front Microbiol.* 2020; 11: 612528. doi: 10.3389/fmicb.2020.612528.
- [43] ElOwnei OAO, Hamid AIO. Effect of storage period on weight loss, chemical composition, microbiological and sensory characteristics of Sudanese white cheese (Gibna Badya). *Pak J Nutr.* 2008; 7(1): 75- 80.
- [44] Frau F, Valdez FG, Pece N. Effect of pasteurization temperature, starter culture, and incubation temperature on the physicochemical properties, yield, rheology, and sensory characteristics of spreadable goat cheese. *J Food Process.* 2014; Volume 2014, Article ID 705746, 8 pages.
- [45] Sabbagh N, Gheisar, HR, Aminlari M. Monitoring the chemical and microbiological changes during ripening of Iranian probiotic low-fat white cheese. *Amer J Anim Vet Sci.* 2010; 5 (4): 249-257.
- [46] Sulejmani E, Pollozhani H, Idrizi XH. Sensory profiling and rheological properties of white brined cheese produced by different starter cultures. *J Hyg Eng Design.* 2011; 1: 309-311.
- [47] Kourkoutas Y, Kandylis P, Panas P, Dooley JSG, Nigam,P, Koutinas AA. (2006). Evaluation of freeze-dried Kefir culture as a starter in Feta-type cheese production. *Appl Environ Microbiol.* 2006; 72(9): 6124-6135.
- [48] Mohammed EHS, Abdalla MOM. Quality evaluation of cooked and uncooked low-salt Sudanese White Soft Cheese (Gibna beyda). *U K J Agric Sci.* 2010; 18(1): 92-104