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TRACKING SHELF-LIFE DETERMINANTS IN SOFT, SEMI-HARD, AND HARD CHEESES: A QUALITY PERSPECTIVE

Mohammed A. Falih[1], Qausar Hamed Alkaisy[1], Ammar B. Altemimi[2],* Tarek Gamal Abedelmaksoud[3]*

[1] Department of Dairy Science and Technology, College of Food Sciences, University of AL-Qasim Green, Al Qasim, Iraq
[2] Department of Food Science, College of Agriculture, University of Basrah, Basrah, Iraq
[3] Food Science Department, Faculty of Agriculture, Cairo University, Giza, Egypt

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cheese quality, HMF, antioxidant activity, microbial safety, storage

ABSTRACT
This study evaluates the physicochemical and microbiological quality indicators of 81 cheese samples (soft, semi-hard, and hard) produced in Iran, Saudi Arabia, and Turkey and marketed in Iraq. Samples were analyzed over storage periods of up to 60 days at 4 °C. Hydroxymethylfurfural (HMF) was quantified using HPLC-DAD, while total viable counts, *Staphylococcus aureus*, *Escherichia coli*, and yeasts/molds were determined by plate count methods. Antioxidant activity and reducing power of water-soluble extracts were assessed using DPPH and ferric reducing assays, respectively. Results showed a wide range of HMF content, with higher values generally observed in semi-hard and hard cheeses. Microbial analysis revealed that soft cheeses had the highest total viable counts and yeast/mold counts, though all values were within permissible limits except for some *E. coli* occurrences. Antioxidant activity and reducing power were highest in hard cheeses, particularly those from Turkey. Storage time significantly influenced moisture content and peroxide values across all cheese types. This study highlights considerable variability in cheese quality across origins and types. Findings support the need for routine quality control and stricter storage guidelines to ensure consumer safety.

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ОТСЛЕЖИВАНИЕ ДЕТЕРМИНАНТОВ СРОКА ГОДНОСТИ В МЯГКИХ, ПОЛУТВЕРДЫХ И ТВЕРДЫХ СЫРАХ: АСПЕКТ КАЧЕСТВА

Фалих М. А.[1], Алкаиси К. Х.[1], Альтемими А. Б.[2],* Абедельмаксуд Т. Г.[3]*

[1] Факультет науки и технологии молока, Колледж пищевых наук, Университет Аль-Касим Грин, Аль-Касим, Ирак
[2] Кафедра пищевых наук, Сельскохозяйственный колледж, Университет Басры, Басра, Ирак
[3] Кафедра пищевых наук, Сельскохозяйственный факультет, Каирский университет, Гиза, Египет

КЛЮЧЕВЫЕ СЛОВА:
качество сыра, ГМФ, антиоксидантная активность, микробиологическая безопасность, хранение

АННОТАЦИЯ
В данном исследовании осуществлена оценка физико-химических и микробиологических показателей качества 81 образца сыра (мягкого, полутвердого и твердого), произведенного в Иране, Саудовской Аравии и Турции, и реализуемого в Ираке. Образцы анализировали в процессе хранения до 60 дней при 4 °C. Проведена количественная оценка гидроксиметилфурфурола (ГМФ) методом ВЭЖХ с использованием детектора на диодной матрице; общее микробное число, количество *Staphylococcus aureus*, *Escherichia coli* и дрожжей/плесеней определяли с использованием метода подсчета колониеобразующих единиц. Антиоксидантную активность и восстановительную способность оценивали с использованием метода на основе DPPH и анализа восстановления железа, соответственно. Результаты показали широкий диапазон содержания ГМФ с более высокими уровнями, обнаруженными в полутвердых и твердых сырах. Микробиологический анализ показал, что мягкие сыры имели наиболее высокие значения общего микробного числа и дрожжей/плесеней, но оставались в рамках допустимых пределов за исключением нескольких случаев обнаружения *E. coli*. Антиоксидантная активность и восстановительная способность были наибольшими в твердых сырах, особенно в сырах из Турции. Продолжительность хранения оказывала значимое влияние на содержание влаги и пероксидное число во всех видах сыра. Данное исследование подчеркивает существенную вариабельность в качестве образцов сыра в зависимости от их происхождения и вида. Результаты подтверждают необходимость регулярного контроля качества и более строгих руководств по хранению для обеспечения безопасности потребителей.

1. Introduction

Cheese is a widely consumed dairy product that provides essential nutrients, including proteins, fats, vitamins, and minerals, as well as a diverse range of bioactive compounds with potential health-promoting properties. The chemical composition, microbial safety, and antioxidant capacity of cheese vary significantly by type (soft, semi-hard, or hard), manufacturing conditions, and storage practices. As cheese is a perishable product, quality deterioration during storage, particularly under refrigeration, can compromise consumer safety and product shelf-life. Therefore, understanding the physicochemical and microbiological changes that occur during cheese storage is critical for ensuring quality and consumer health. One of the interesting compounds that form during heat treatment or storage of dairy products is 5-hydroxymethylfurfural (HMF). HMF is a marker of heat-induced reactions, particularly the Maillard reaction and sugar dehydration, and its accumulation is associated with potential health risks, including genotoxicity and mutagenicity [1,2]. Although HMF is regulated in products such as honey and fruit juice, its presence in cheese is less studied and not yet legislatively controlled, despite evidence that heat treatment and prolonged storage can elevate HMF concentrations [1].

Equally important is the microbial quality of cheese. Cheese can harbor various microorganisms, including spoilage bacteria and foodborne pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and, in rare cases, *Salmonella*. These contaminants may originate from raw milk, unhygienic processing, or post-processing contamination [3,4]. While certain microorganisms are essential for flavor development in cheese,

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uncontrolled microbial growth poses serious safety risks. Thus, periodic monitoring of microbial indicators is crucial, particularly during storage, to evaluate hygiene, shelf-life, and overall safety [3].

Additionally, cheese possesses antioxidant properties owing to peptides, amino acids, vitamins, and fatty acids. This antioxidant activity is associated with neutralizing free radicals and reducing oxidative stress, thereby providing additional health benefits [5]. The antioxidant profile of cheese can change during storage due to proteolysis and microbial activity, leading to the release of bioactive peptides with radical-scavenging and metal-reducing capacities [6,7].

Despite the importance of these parameters, few comparative studies have evaluated the chemical and microbiological quality of cheese produced in different countries under standardized storage conditions. This is particularly relevant in markets where cheeses imported from neighboring countries are widely available. Differences in manufacturing practices, heat treatment, and hygiene standards between these countries can lead to considerable variations in product quality.

Given this context, the present study examines the physicochemical characteristics, microbial quality, and antioxidant potential of soft, semi-hard, and hard cheeses from three different countries Iran, Saudi Arabia, and Turkey. This study aims to assess and compare the quality and safety of commercially available cheeses by monitoring changes in HMF content, moisture, peroxide value, microbial counts, antioxidant activity, and reducing power during refrigerated storage (4 °C), using validated analytical techniques such as HPLC, the DPPH assay, and microbiological plating methods.

2. Objects and methods

2.1. Sample collection

A total of 27 cheese samples were collected, with three replicates per type, resulting in 81 individual samples representing all cheese categories included in the study. The selected cheeses were commercially available products from various brands sold in markets and stores across Babylon Governorate, Iraq, from November 2024 to February 2025. The cheese samples from three countries: Iran, Saudi Arabia, and Turkey (A, B, and C, respectively) were categorized into three main types: soft, semi-hard, and hard cheese. All samples were assigned alphanumeric codes that indicated three attributes: cheese type, the sample number within each category (1–3), and the country of origin. Soft cheeses were labeled with the prefix CHS, semi-hard cheeses with CHM, and hard cheeses with CHH. The letters I, S, and T represented cheeses from countries A, B, and C, respectively.

Accordingly, the soft cheese samples were designated as CHS1-I, CHS2-I, CHS3-I (country A); CHS1-S, CHS2-S, CHS3-S (country B); and CHS1-T, CHS2-T, CHS3-T (country C). The semi-hard samples included CHM1-I, CHM2-I, CHM3-I (country A); CHM1-S, CHM2-S, CHM3-S (country B); and CHM1-T, CHM2-T, CHM3-T (country C). The hard cheese samples were labeled as CHH1-I, CHH2-I, CHH3-I (country A); CHH1-S, CHH2-S, CHH3-S (country B); and CHH1-T, CHH2-T, CHH3-T (country C).

During sample selection, strict criteria were applied to ensure that the samples reflected the consumption of popular brands across different age groups and income levels, while also accounting for product availability during the study period.

To standardize the starting point for comparative analysis across the different cheese types, a “zero-time” reference point was established for all baseline analyses prior to storage. This standardization was based on production dates, with no more than five days elapsed from production date for any of the soft cheese samples, given their high perishability. For semi-hard and hard cheeses, where post-production market availability can vary, the zero-time point was defined so that selected samples differed in production date by no more than 15 days. This 15-day window was chosen because the lower moisture and higher stability of semi-hard and hard cheeses minimize significant physicochemical or microbial changes during this period, ensuring baseline consistency [8]. For example, samples available two months and two months plus 10 days after production were considered to fall within the same zero-time window. This reference point served as a unified baseline for evaluation, ensuring consistency in comparisons across cheese types and countries of origin and improving the accuracy of subsequent storage-related assessments.

2.2. Determination of Hydroxymethylfurfural (HMF)

A 0.5 g portion of the cheese sample was placed into a 50 mL centrifuge tube. To this, 9 mL of ultrapure water (containing 0.1 % formic acid) was added, followed by 0.5 mL of Carrez I solution (potassium ferrocyanide) and 0.5 mL of Carrez II solution (zinc acetate). The mixture was homogenized for 1 minute and then centrifuged at 4.000 rpm for 10 minutes at 4 °C (Anezka T 30B centrifuge, Janetzki and Schnuch AG, Germany). The supernatant was transferred to a new 50 mL centrifuge tube. An additional 5 mL of ultrapure water was added to the residue, followed by homogenization and centrifugation as previously described. This step was repeated once more, collecting the upper liquid layer (supernatant) each time.

From the pooled supernatant, 1 mL was transferred to a 1.5 mL Eppendorf tube and centrifuged at 14,000 rpm for 10 minutes at 4 °C (MSB010. CX1.5, Gallenkamp, England). The resulting supernatant was filtered through a 0.2 µm membrane filter [9,10].

For HPLC analysis, 20 µL of the filtered extract was injected directly into the system. An HPLC-DAD system (Azura, Knauer, Germany) equipped with an automatic injector (D1357) and a diode array detector (DAD 2.1L) was used. Detection was performed at 285 nm. The mobile phase consisted of water: methanol (10 : 90 v/v) at a flow rate of 1 mL/min. Separation was achieved using a C18 column (Shimpack, Shimadzu, Japan) measuring 4.6 × 250 mm. A standard calibration curve for HMF was prepared at concentrations of 0, 0.05, 0.5, 5, 25, and 50 µg/mL.

2.3. Microbiological analysis of cheese samples

Prior to conducting the microbiological tests, 10 g of each cheese sample was homogenized in 90 mL of sterile 2 % (w/v) sodium citrate solution. Serial decimal dilutions were then prepared using sterile 0.1 % peptone water. Microbiological analysis was performed by enumerating the following microorganisms as described by Hernández-Morales et al. [11] and Rashtchi et al. [12]: Total viable count was determined on nutrient agar. *Staphylococcus aureus* was enumerated on Mannitol Salt Agar (MSA). The medium was sterilized at 121 °C for 15 minutes (Systec: yx-280b, Italy). The samples were inoculated using the pour plate technique and incubated at 37 °C for 24 hours (FAV 061, Memmert, England). Colony-forming units were expressed as log cfu/g for all cheese samples [13]. *Escherichia coli* was detected on MacConkey agar using the pour plate method. Samples were incubated at 37 °C for 24 hours (FAV 061, Memmert, England), and colonies were then counted [14]. *Salmonella* spp. detection was carried out by weighing 1 g (Ohauscalaxy Germany) of each cheese sample and pre-enriching it in Tetrathionate broth, followed by incubation at 37 °C for 24 hours (FAV 061, Memmert, England). One milliliter of the first dilution was then plated onto Salmonella-Shigella agar, and incubated at 37 °C for 24 hours (FAV 061, Memmert, England). Grown colonies were counted accordingly [15]. Yeasts and molds were enumerated on Potato Dextrose Agar (PDA) prepared by dissolving 39 g in 1 L of water and sterilized at 121 °C for 15 minutes (Systec: yx-280b, Italy). Chloramphenicol was prepared by dissolving 1 g in 10 mL of 70 % ethanol, and 1 mL of the prepared solution was added per liter of PDA. The pour plate technique was used for inoculation, and plates were incubated at 25 °C for 5–7 days (FAV 061, Memmert, England) [16].

2.4. Preparation of water-soluble extracts (WSEs) of cheese

Twenty grams of freeze-dried cheese samples were ground into a fine powder and mixed with 180 mL of distilled water. The mixture was then sonicated in an ultrasonic water bath (Model LUC-405, Labocon, United Kingdom) for 60 minutes at 40 °C. After cooling, the water-soluble extract (WSE) was centrifuged at 6000 × g for 20 minutes at 4 °C (Anezka T 30B centrifuge, Janetzki and Schnuch AG, Germany) to remove the fat layer. The supernatant was filtered through Whatman No. 2 filter paper, followed by additional filtration through a 0.22 µm membrane filter to remove residual impurities. The clarified WSE was stored at −20 °C until further analyses were performed [17].

2.5. Antioxidant activity determination

The antioxidant activity of the water-soluble extracts (WSEs) was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, as described by Uzunsoy [18]. A volume of 1.5 mL of WSE was mixed with 1.5 mL of freshly prepared DPPH solution (60 µM in 95 % methanol) and incubated in the dark at room temperature for 45 minutes. Absorbance was then measured at 517 nm using a UV-visible spectrophotometer (SP-3000nano, Optima, Japan). The radical- scavenging activity of the WSEs was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = [1 - (A_{WSE}/A_B)] \times 100, \quad (1)$$

where A_B is the absorbance of the blank (deionized water); A_{WSE} is the absorbance of the sample (Water-Soluble Extract, WSE).

2.6. Reducing power assay

The reducing power assay was conducted according to the method described by Ramos et al. [19] with slight modifications. A volume of 250 µL of the water-soluble extract (WSE) was mixed with 250 µL of phosphate buffer (0.2 M, pH 6.6) and 250 µL of potassium ferricyanide (1 %). The mixture was incubated at 50 °C for 20 minutes (FAV 061, Memmert, England). Subsequently, 250 µL of trichloroacetic acid (10 %) was added, and the mixture was centrifuged at 3000 rpm for 10 minutes (Model: PLC-036H, Labnet, USA). From the resulting supernatant, 500 µL was collected and mixed with 400 µL of distilled water and 100 µL of ferric chloride (0.1 %). The final mixture was incubated at 50 °C for 10 minutes. Absorbance was measured at 700 nm using a UV-visible spectrophotometer (SP-3000nano, Optima). The reducing power of the sample was calculated as follows:

$$(\%)\ \text{Reducing power} = [(Abs_M - Abs_B)/Abs_M] \times (100), \qquad (2)$$

where Abs_M is the absorbance of the sample; Abs_B is the absorbance of the blank.

2.7. Storage evaluation

Storage tests were conducted at 4 °C for different cheese types: soft cheeses were evaluated at 0, 7, 14, 21, and 28 days of storage, while semi-hard and hard cheeses were evaluated at 0, 15, 30, 45, and 60 days.

2.8. Determination of moisture content

The moisture content of the cheese samples was determined by drying the samples in an oven at 105 °C (Schutzart-Din 4005 D-IP, Germany) until a constant weight was achieved. The moisture content was calculated based on weight loss, according to the method described by Cebeci et al. [20].

2.9. Determination of peroxide value

The peroxide value was determined according to the method of Saad et al. [21]. Briefly, 5 g of cheese was dissolved in 30 mL of glacial acetic acid/chloroform mixture (3:2, v/v). Then, 1 mL of saturated potassium iodide solution and 40 mL of distilled water were added. The mixture was titrated with 0.1 N sodium thiosulfate ($Na_2S_2O_3$) until the yellow color disappeared. Next, 0.5 mL of 1 % starch solution was added, and titration continued until the blue color disappeared.

The peroxide value was calculated using the following equation:

$$\text{Peroxide value (mEq/kg sample)} = S \times N \times 1000 / W, \qquad (3)$$

where S is the volume of sodium thiosulfate solution, mL; N is the normality of the sodium thiosulfate solution; W is the weight of the cheese sample, g.

2.10. Statistical analysis

The data were statistically analyzed using a two-way analysis of variance (Two-Way ANOVA) at $\alpha = 0.05$, followed by the Least Significant Difference (LSD) test for multiple comparisons. This was done to evaluate the effects of the two independent variables — cheese type and country of manufacture — as well as their first-order interaction. The analysis was performed using SPSS software, version 13 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Determination of Hydroxymethylfurfural (HMF)

Figure 1 presents the chromatographic profiles of hydroxymethylfurfural (HMF) standard alongside those of the cheese samples. These profiles reveal varying HMF concentrations across the samples. Table 1 displays the quantified HMF content in the tested cheese samples, with cheese samples from country A containing HMF in the range of-ND (Not Detected) to 1.8410 µg/g. In cheese samples from country B, HMF levels ranged from ND to 1.7856 µg/g, while country C samples exhibited concentrations ranging from ND to 1.4436 µg/g.

These findings are consistent with those reported by Li et al. [1], who evaluated four furfural compounds, including 5-hydroxymethylfurfural (5-HMF), in various cheese types available in the Chinese market. Their results demonstrated significantly higher concentrations of furfural compounds, especially 5-HMF, in semi-hard and hard cheeses compared to soft and fresh varieties. The researchers attributed these differences to heat processing of milk prior to cheesemaking, which may influence HMF formation. This supports the hypothesis that HMF could serve as a reliable indicator for assessing heat processing intensity [22].

Given the potential toxicity of HMF, its concentration in foods must be regulated. Current regulations by the Codex Alimentarius and the European Union specify a maximum HMF limit only for honey, ranging from 40 to 80 mg/kg, depending on the country of origin [23,24]. Similarly, the International Federation of Fruit Juice Producers [25] recommends a maximum concentration of 10 mg/L in fruit juices and 25 mg/kg in fruit juice concentrates. Additionally, the Codex standard [24] considers a concentration of 25 mg/kg in apple juice as a marker of degradation and/or excessive heat treatment.

Despite its toxicological importance, no maximum allowable HMF concentration has been established for other food categories [26–28]. According to Xing et al. [29], HMF levels in dairy products ranged from 37 to 891 mg/kg as measured by HPLC-DAD, although other studies have reported varying concentrations across dairy products.

3.2. Analysis of microbiological indicators

Table 2 shows the bacterial counts in the cheese samples. ANOVA results revealed significant differences ($p < 0.05$) in bacterial counts across cheese types and countries of origin, including a significant interaction effect.

The average total bacterial count was as follows: cheeses from county A, 3.595 log CFU/g; cheeses from country B, 3.357 log CFU/g; and cheeses from country B, 3.521 log CFU/g. When categorized by cheese type, the total bacterial count was highest in soft cheeses (4.226 log CFU/g), followed by semi-hard cheeses (3.458 log CFU/g) and hard cheeses (2.788 log CFU/g). All values were below the maximum permissible limit of 5.47 log CFU/g as defined by the European Commission [30].

Table 2 also highlights the presence of yeasts and molds in cheese samples from all three countries. ANOVA confirmed significant differences ($p < 0.05$) in yeast and mold counts based on cheese type and origin, including a significant interaction effect. Generally, cheeses from Country C exhibited higher contamination levels compared to those from Countries B and A. Regarding cheese type, soft cheeses contained significantly higher yeast and mold populations than semi-hard and hard varieties. Notably, the mean count in soft cheeses approached the acceptable limit of 2 log CFU/g defined by the standard [14], whereas counts in semi-hard and hard cheeses remained well below this threshold.

Airborne contamination, production equipment, and plastic packaging films are major sources of mold contamination in cheeses. Airborne transmission is particularly critical, as the presence of molds negatively affects the sensory qualities of cheese and poses a potential health risk due to their ability to produce mycotoxins [31].

The results also confirmed the presence of Staphylococcus spp. in some soft and semi-hard cheese samples from the three countries, while the bacterium was not detected in any of the hard cheeses. ANOVA results showed significant differences ($p < 0.05$) between brands based on cheese type and origin, with a notable interaction effect. Although *Staphylococcus* counts varied among the three countries, with Country C exhibiting notably higher average counts than Countries A and B, all recorded levels remained significantly below the maximum permissible limit of 2 log CFU/g [32,33].

Contamination by Staphylococcus aureus may occur via three main pathways: presence in raw milk, cross-contamination from infected handlers, and contact with contaminated surfaces or equipment. Inadequate

Table 1. **Hydroxymethylfurfural (HMF) content (µg/g) in cheese samples**

Таблица 1. **Содержание гидроксиметилфурфурола (ГМФ) (мкг/г) в образцах сыра**

Cheese type / Country of origin	Peak area, %	Concentration, µg/ml	Sample volume, ml	Sample weight, g	Final concentration (µg/g)
CHH2-I	105.206	0.7671 ± 0.06	1.2	0.5	1.8410 ± 0.07
CHH3-T	100.142	0.7218 ± 0.05	1.0	0.5	1.4436 ± 0.09
CHM1-S	102.396	0.7440 ± 0.06	1.2	0.5	1.7856 ± 0.07
CHM2-T	94.241	0.6684 ± 0.03	0.9	0.5	1.2032 ± 0.06
CHS2-T	49.006	0.2543 ± 0.04	0.8	0.5	0.4069 ± 0.08
CHS1-I	37.531	0.1493 ± 0.06	1.0	0.5	0.2987 ± 0.03
CHS2-S	38.737	0.1603 ± 0.04	0.8	0.5	0.2565 ± 0.04

* Soft cheese samples: CHS1-I, CHS2-I, CHS3-I (country A); CHS1-S, CHS2-S, CHS3-S (country B); and CHS1-T, CHS2-T, CHS3-T (country C). Semi-hard samples: CHM1-I, CHM2-I, CHM3-I (country A); CHM1-S, CHM2-S, CHM3-S (country B); and CHM1-T, CHM2-T, CHM3-T (country C). Hard cheese samples: CHH1-I, CHH2-I, CHH3-I (country A); CHH1-S, CHH2-S, CHH3-S (country B); and CHH1-T, CHH2-T, CHH3-T (country C).

Table 2. **Results of microbiological analysis of the cheese samples**

Таблица 2. **Результаты микробиологического анализа образцов сыра**

Cheese sample	Total viable count, log CFU/g	*S. aureus*, log CFU/g	*Salmonella*	*E. coli*, log CFU/g	Yeasts and molds, log CFU/g
			Soft cheeses		
CHS1-I	4.11±0.02	1.47±0.04	ND	1.49±0.08	1.82±0.09
CHS2-I	4.62±0.04	1.39±0.04	ND	1.43±0.06	1.91±0.05
CHS3-I	4.41±0.04	ND	ND	1.50±0.05	2.62±0.07
CHS1-S	4.12±0.06	ND	ND	1.38±0.07	2.51±0.05
CHS2-S	3.91±0.03	ND	ND	1.43±0.09	ND
CHS3-S	4.31±0.05	1.39±0.06	ND	ND	2.82±0.09
CHS1-T	4.23±0.04	1.39±0.07	ND	ND	1.81±0.06
CHS2-T	3.81±0.03	1.60±0.08	ND	ND	1.73±0.09
CHS3-T	4.52±0.08	1.00±0.05	ND	1.51±0.09	2.20±0.07
L.S.D (Cheese type)	0.260	0.155	—	0.045	0.500
L.S.D (Country of origin)	0.194	0.374	—	0.434	0.136
			Semi-hard cheeses		
CHM1-I	3.54±0.07	ND	ND	ND	ND
CHM2-I	3.49±0.04	ND	ND	ND	ND
CHM3-I	3.49±0.07	ND	ND	ND	ND
CHM1-S	3.30±0.09	ND	ND	ND	1.54±0.04
CHM2-S	3.54±0.05	1.39±0.07	ND	1.44±0.07	1.60±0.07
CHM3-S	3.54±0.05	ND	ND	1.53±0.05	1.77±0.09
CHM1-T	3.59±0.05	ND	ND	1.34±0.09	1.72±0.04
CHM2-T	3.32±0.09	1.39± 0.05	ND	ND	1.81±0.07
CHM3-T	3.32±0.08	1.39±0.08	ND	ND	ND
L.S.D (Cheese Type)	NS	0.461	—	0.030	0.050
L.S.D (Country of origin)	0.043	0.463	—	0.466	0.460
			Hard cheeses		
CHH1-I	3.00±0.07	ND	ND	ND	ND
CHH2-I	2.80±0.08	ND	ND	ND	ND
CHH3-I	2.90±0.04	ND	ND	ND	1.72±0.06
CHH1-S	2.70±0.09	ND	ND	ND	ND
CHH2-S	2.50±0.08	ND	ND	ND	ND
CHH3-S	2.30±0.04	ND	ND	ND	1.30±0.07
CHH1-T	2.90±0.05	ND	ND	ND	1.40±0.07
CHH2-T	3.00±0.04	ND	ND	ND	1.60±0.09
CHH3-T	3.00±0.09	ND	ND	ND	1.81±0.05
L.S.D (Cheese type)	0.048	—	—	—	0.066
L.S.D (Country of origin)	0.072	—	—	—	0.140
			Averages		
Soft cheeses avg.	4.226	0.915	—	0.971	2.177
Semi-hard cheeses avg.	3.458	0.463	—	0.538	0.937
Hard cheeses avg.	2.788	—	—	—	0.870
Country A avg.	3.595	0.317	—	0.491	0.896
Country B avg.	3.357	0.308	—	0.642	1.282
Country C avg.	3.521	0.752	—	0.316	1.564

* Results as mean±SD (standard deviation); ND: Not Detected; L.S.D.: Least Significant Difference; Soft cheese samples: CHS1-I, CHS2-I, CHS3-I (country A); CHS1-S, CHS2-S, CHS3-S (country B); and CHS1-T, CHS2-T, CHS3-T (country C). Semi-hard samples: CHM1-I, CHM2-I, CHM3-I (country A); CHM1-S, CHM2-S, CHM3-S (country B); and CHM1-T, CHM2-T, CHM3-T (country C). Hard cheese samples: CHH1-I, CHH2-I, CHH3-I (country A); CHH1-S, CHH2-S, CHH3-S (country B); and CHH1-T, CHH2-T, CHH3-T (country C).

pasteurization or the use of low-quality starter cultures may also contribute to contamination [34].

Table 2 indicates the presence of *Escherichia coli* in some cheese samples. ANOVA analysis revealed significant differences ($p < 0.05$) between brands across cheese types and countries of origin, with a significant interaction effect. These results suggest a correlation between cheese texture and bacterial contamination, as *E. coli* was predominantly found in soft cheeses, to a lesser extent in semi-hard cheeses, and was absent in hard cheeses.

The contamination levels were evaluated against GSO standards [33], which specify maximum limits of 1 log CFU/g for soft cheeses and 0 log CFU/g for semi-hard cheeses. While most samples adhered to these limits, samples CHM2-S, CHM3-S, and CHM1-T exceeded the permissible thresholds by 1.44, 1.53, and 1.34 log CFU/g, respectively. Notably, these exceedances were significant even when accounting for measurement uncertainty and method-related errors. All hard cheese samples from the three countries were free from *E. coli*, in compliance with the standards [33,34].

The elevated *E. coli* counts observed in some samples reflect potential deficiencies in hygiene and sanitation during manufacturing and handling. As a fecal contamination indicator, the presence of *E. coli* is of serious concern, underscoring the need for strict quality control and adherence to food safety protocols throughout the production and supply chain.

Finally, *Salmonella* spp. was not detected in any of the cheese samples, meeting the requirements of absence in 25 grams as specified by the standard [32,34].

3.3. Determination of antioxidant activity

Table 3 presents the antioxidant activity of extracts obtained from the cheese samples. The results indicate a distinct trend based on texture, with hard cheeses exhibiting the highest antioxidant activity, followed by semi-hard and soft cheeses. While significant differences were observed between cheeses from countries A and B and between B and C, no significant difference was found between countries A and C. Statistical analysis confirmed that both cheese type and country of origin significantly influenced antioxidant activity ($p < 0.0001$), with a significant interaction between these two factors.

Revilla et al. [35] and Kose and Ocak [36] attributed variations in antioxidant activity to milk type, processing methods, and storage periods. Their findings demonstrated a gradual increase in antioxidant potential over time due to ongoing proteolysis, which leads to the release of soluble peptides and sulfur-containing amino acids. In contrast to these consistent trends regarding storage time, other studies have reported divergent results at specific time points. For instance, Al-Hamdani et al. [37] observed high antioxidant activity (63 %) in soft white cheese stored for 21 days, whereas no antioxidant activity was detected

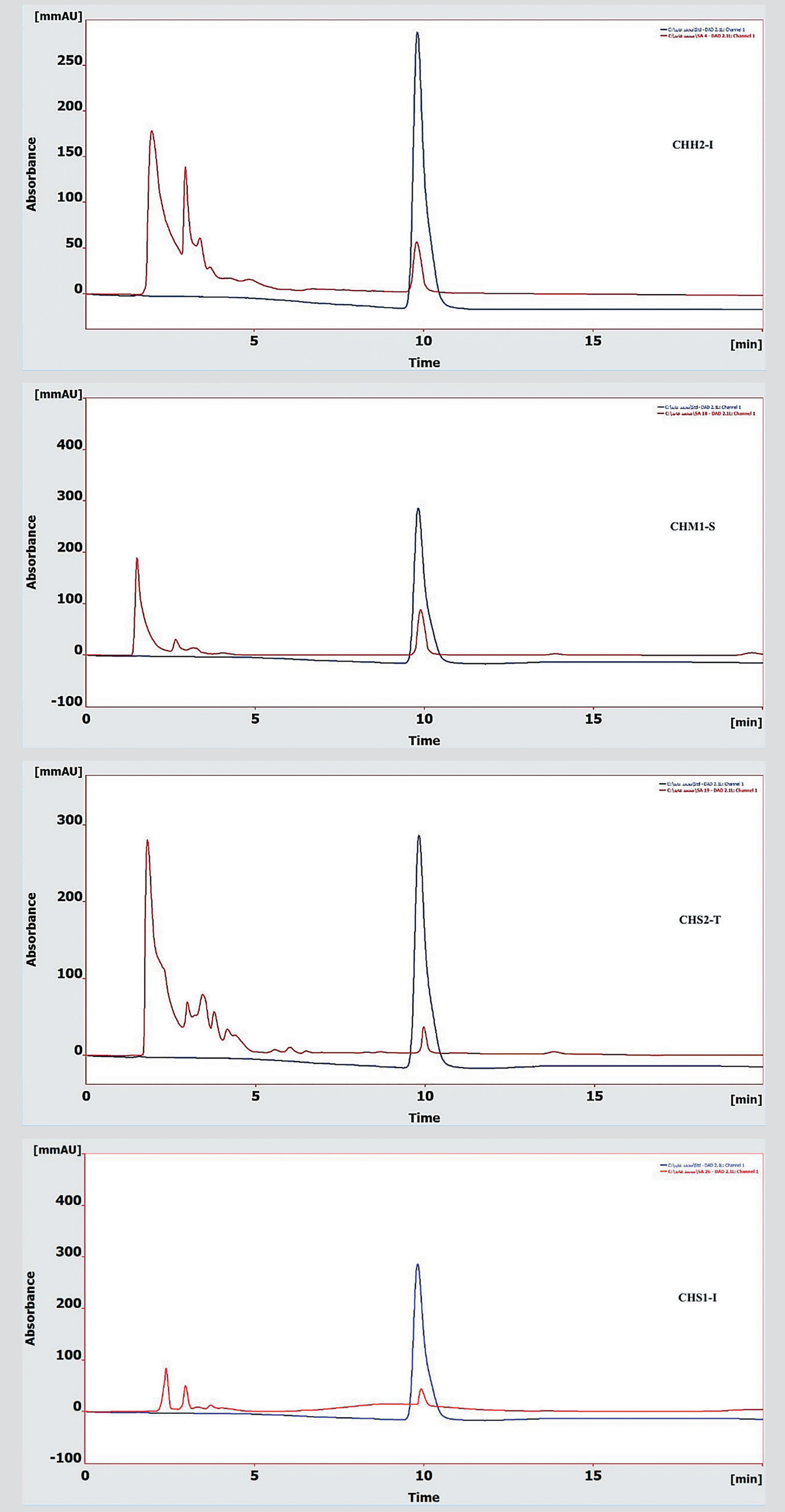

Figure 1. **HPLC chromatograms of hydroxymethylfurfural (HMF) standard and cheese samples**
Рисунок 1. **ВЭЖХ хроматограммы стандартного образца гидроксиметилфурфурола (ГМФ) и образцов сыра**

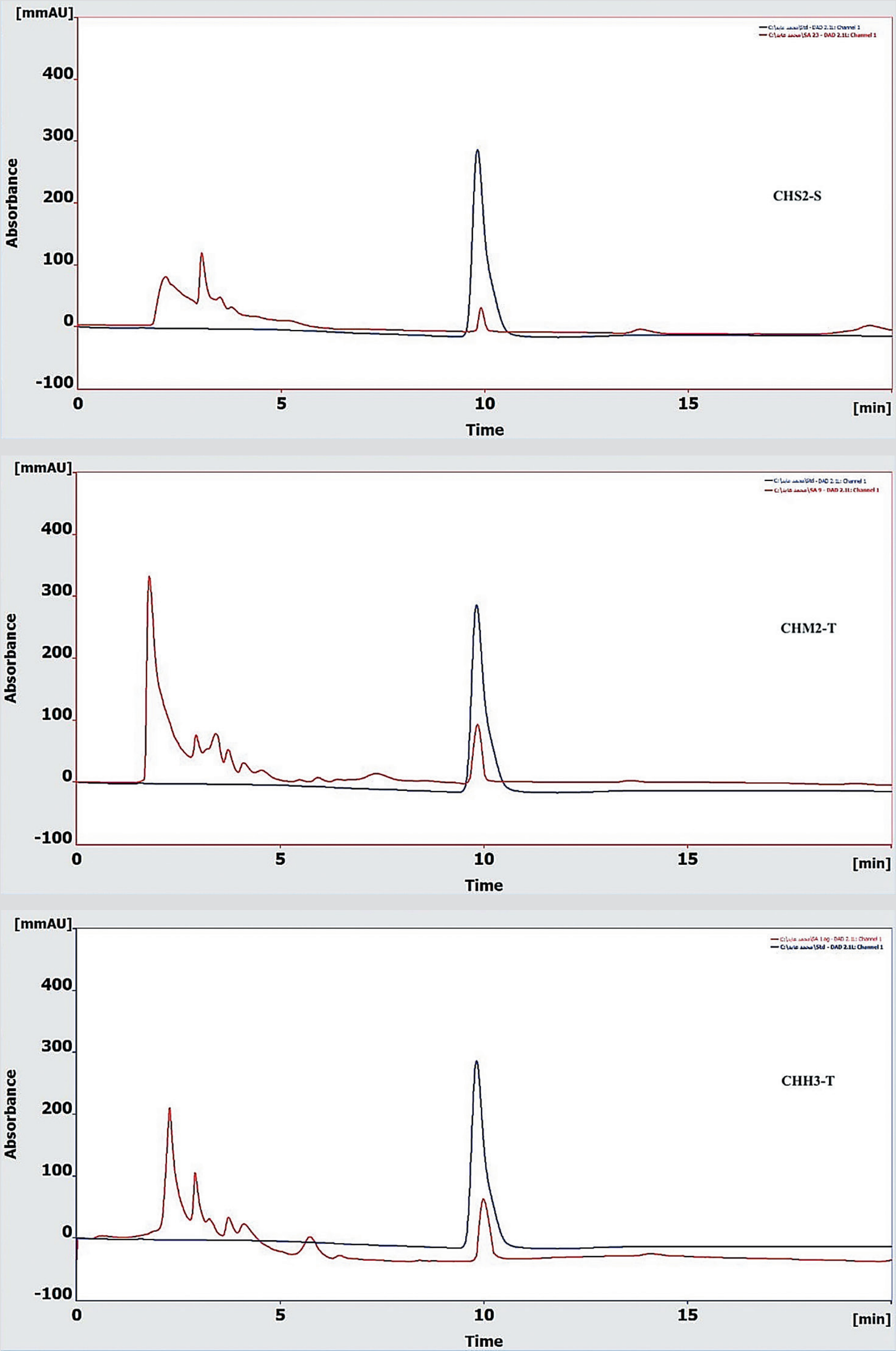

Figure 1 (end). **HPLC chromatograms of hydroxymethylfurfural (HMF) standard and cheese samples**

Рисунок 1 (окончание). **ВЭЖХ хроматограммы стандартного образца гидроксиметилфурфурола (ГМФ) и образцов сыра**

in extracts of full-fat and low-fat soft cheese in other research [38]. Given that the soft cheeses in the present study were analyzed after 28 days at 4 °C, the lower activity observed here (compared to Al-Hamdani) suggests that specific compositional differences, rather than storage duration, were primarily responsible for the variation. The antioxidant activity values for semi-hard cheeses in the present study were notably higher than the 23.35 ± 0.82 % reported by Uzunsoy [18] for Camis cheeses after 30 days of storage. This increase is consistent with the effect of ripening time, as the semi-hard cheeses in this study were stored for 60 days at 4 °C, allowing for greater proteolysis compared to the 30-day period in Uzunsoy's study. Regarding hard cheeses, the findings of the current study align closely with those of Chen et al. [39], who reported approximately 47 % activity for Cheddar cheese. It is important to note that Chen et al. observed these results after a substantially longer storage period of 16 weeks (112 days), whereas the hard cheeses in the present study reached comparable levels after only 60 days. This indicates that the hard cheeses in this study underwent rapid protein degradation and release of antioxidant peptides within the first two months of storage.

3.4. Determination of reducing power

Table 3 shows the reducing power values of the cheese samples. The mean reducing power values were 42.544 ± 8.194 % for cheeses from country A, 42.22 ± 11.686 % for cheeses from country B, and 44.231 ± 12.031 % for cheeses from country C. Regarding cheese types, the mean reducing power was 31.252 ± 2.509 % for soft cheeses, 42.587 ± 2.281 % for semi-hard cheeses, and 55.155 ± 4.142 % for hard cheeses.

The lowest reducing power was observed in sample CHS1-I at 27.21 ± 0.567 %, while the highest was found in the hard cheese sample from country C (CHH1-T), at 60.56 ± 0.523 %.

Statistical analysis indicated significant effects of both cheese type and country of origin, along with a significant first-order interaction between these variables. Significant differences ($p < 0.05$) in reducing power were observed between cheeses from countries A and C and between cheeses from countries B and C, while no significant differences were found between cheeses from countries A and B ($p > 0.05$).

Ramos et al. [19] reported that bioactive compounds in cheese extracts possess reducing power, with the ability to donate electrons and neutralize oxidative intermediates generated during lipid oxidation. This property enables them to act as both primary and secondary antioxidants. Jafari et al. [40] observed that cheese samples exhibited higher reducing power compared to other dairy products, potentially due to a lower electrode potential (Eh) for redox reactions. While milk typically has an Eh of approximately +150 mV, cheese exhibits an Eh of around −250 mV. Although the precise mechanisms underlying this reduction remain

Table 3. **Antioxidant activity and reducing power (%) in cheese samples under study**

Таблица 3. **Антиоксидантная активность и восстановительная способность (%) в исследуемых образцах сыра**

Cheese type / Country of origin	Antioxidant activity, % ± SD	Reducing power, % ± SD
Soft cheeses		
CHS1-I	24.262 ± 0.143	30.46 ± 0.759
CHS2-I	25.117 ± 0.113	33.11 ± 1.018
CHS3-I	28.078 ± 0.154	36.14 ± 1.109
CHS1-S	28.386 ± 0.588	27.21 ± 0.567
CHS2-S	27.740 ± 0.514	30.51 ± 0.986
CHS3-S	28.424 ± 0.314	29.44 ± 0.575
CHS1-T	28.921 ± 0.212	31.47 ± 0.674
CHS2-T	29.565 ± 0.154	29.42 ± 0.560
CHS3-T	30.869 ± 1.324	33.51 ± 0.511
Semi-hard cheeses		
CHM1-I	40.435 ± 0.929	40.62 ± 0.690
CHM2-I	40.121 ± 0.815	44.41 ± 0.502
CHM3-I	40.642 ± 1.039	45.11 ± 0.940
CHM1-S	35.382 ± 0.573	39.76 ± 0.120
CHM2-S	36.604 ± 0.154	40.78 ± 0.225
CHM3-S	34.535 ± 0.149	45.32 ± 0.367
CHM1-T	37.588 ± 0.605	40.21 ± 0.450
CHM2-T	38.525 ± 0.148	42.57 ± 0.228
CHM3-T	35.352 ± 0.147	44.51 ± 0.407
Hard cheeses		
CHH1-I	49.659 ± 1.714	55.62 ± 0.441
CHH2-I	47.583 ± 1.487	48.32 ± 0.510
CHH3-I	50.529 ± 0.553	49.11 ± 0.890
CHH1-S	45.328 ± 0.186	53.64 ± 0.390
CHH2-S	46.046 ± 0.155	55.73 ± 0.396
CHH3-S	47.285 ± 0.648	57.59 ± 0.566
CHH1-T	52.118 ± 0.223	60.56 ± 0.523
CHH2-T	50.606 ± 0.227	58.54 ± 0.445
CHH3-T	46.617 ± 1.179	57.29 ± 0.804
Averages by cheese type		
Soft cheeses	27.929 ± 2.066	31.252 ± 2.509
Semi-hard cheeses	37.687 ± 2.366	42.587 ± 2.281
Hard cheeses	48.419 ± 2.368	55.155 ± 4.142
Averages by country		
Cheeses from country A	38.492 ± 3.319	42.544 ± 8.194
Cheeses from country B	36.637 ± 2.526	42.220 ± 11.686
Cheeses from country C	38.870 ± 4.418	44.231 ± 12.031
Significance of variables and interactions		
Cheese type	$p < 0.0001$	$p < 0.0001$
Country of origin	$p < 0.0001$	$p < 0.0001$
Cheese type × Country interaction	$p < 0.0001$	$p < 0.0001$

* *p-value* at 0.05; Results as mean ± SD (standard deviation); Soft cheese samples: CHS1-I, CHS2-I, CHS3-I (country A); CHS1-S, CHS2-S, CHS3-S (country B); and CHS1-T, CHS2-T, CHS3-T (country C). Semi-hard samples: CHM1-I, CHM2-I, CHM3-I (country A); CHM1-S, CHM2-S, CHM3-S (country B); and CHM1-T, CHM2-T, CHM3-T (country C). Hard cheese samples: CHH1-I, CHH2-I, CHH3-I (country A); CHH1-S, CHH2-S, CHH3-S (country B); and CHH1-T, CHH2-T, CHH3-T (country C).

unclear, it is generally attributed to lactose fermentation into lactic acid by starter bacteria and the partial reduction of oxygen to water.

Sarić et al. [41] also demonstrated that Kupres cheese, a semi-hard variety, exhibited reducing power. Key contributors to this property include free amino acids, low- and high-molecular-weight peptides, and proteins, all of which are effective at scavenging free radicals. The antioxidant activity of peptides is influenced by factors such as radical scavenging ability, metal ion binding capacity, and ferric ion reduction, which depend on the peptide's amino acid composition, molecular weight, physical structure, content of sulfhydryl-containing amino acids (e.g., cysteine and methionine), aromatic residues (e.g., tryptophan, tyrosine, and phenylalanine), histidine content, and hydrophobicity.

Furthermore, differences in reducing power among cheeses may be attributed to the content of compounds derived from milk and those generated by microbial activity. Milk-derived antioxidants include α-tocopherol, ascorbic acid, whey proteins, caseins, and minerals. Additionally, although fatty acids are not direct antioxidants, they may contribute indirectly to reducing power by interacting with other antioxidants (e.g., vitamin E and β-carotene). Microbial metabolites such as folic acid, known for its antioxidant capacity and ability to reduce Fe^{3+} to Fe^{2+}, may also enhance cheese's reducing power [42].

The values obtained in the present study were notably higher than those reported by Ramos et al. [19], who found that the reducing power of Gouda cheese was 11.2 % after 90 days of storage at 10 °C.

3.5. Effect of storage duration on moisture content

Figure 2A illustrates the impact of storage duration on the moisture content of the soft cheese samples, stored for 28 days at 4 °C. The moisture content of the soft cheese samples was 56.0 ± 2.5, 62.3 ± 2.3, 60.3 ± 2.6, 54.8 ± 2.5, 55.3 ± 2.8, 55.0 ± 2.51, 58.4 ± 2.4, 55.1 ± 2.5, and 61.0 ± 2.3 % for CHS1-I, CHS2-I, CHS3-I, CHS1-S, CHS2-S, CHS3-S, CHS1-T, CHS2-T, and CHS3-T, respectively. The greatest reduction in moisture content was observed in CHS1-S, with a 14.10 % decrease after 28 days of storage at 4 °C. Statistical analysis revealed that both cheese type-and country of origin, as well as their interaction, had significant effects on moisture content.

Figure 2B shows the effect of storage time on the moisture content of the semi-hard cheese samples, stored for 60 days at 4 °C. The moisture content was 43.77 ± 2.5, 40.26 ± 2.3, 42.76 ± 2.6, 39.96 ± 2.5, 43.56 ± 2.8, 43.76 ± 2.51, 48.66 ± 2.4, 39.46 ± 2.5, and 40.06 ± 2.3 % for CHM1-I, CHM2-I, CHM3-I, CHM1-S, CHM2-S, CHM3-S, CHM1-T, CHM2-T, and CHM3-T, respectively. The most significant moisture loss, 22.14 %, was observed in CHM2-T after 60 days of storage at 4 °C.

Figure 2C displays the effect of storage duration on the moisture content of the hard cheese samples, stored for 60 days at 4 °C. The moisture contents were 37.31 ± 2.2, 35.36 ± 5.2, 36.35 ± 2.6, 34.24 ± 1.3, 33.34 ± 5.2, 27.59 ± 4.2, 36.41 ± 4.2, 38.19 ± 7.2, and 37.48 ± 8.2 % for CHH1-I, CHH2-I, CHH3-I, CHH1-S, CHH2-S, CHH3-S, CHH1-T, CHH2-T, and CHH3-T, respectively. The greatest reduction in moisture content was observed in CHH2-T, with a 25.4 % decrease after 60 days of storage at 4 °C.

These findings are consistent with those reported by Perveen et al. [43], who noted a reduction in moisture content during refrigerated storage of cheese. This moisture loss could be attributed to structural protein breakdown (syneresis), leading to water expulsion. Additionally, variations in water activity, salt content, and protein interactions contribute to the gradual reduction in the water-holding capacity of cheese over time.

3.6. Effect of storage duration on peroxide value

Table 4 presents the effect of storage duration on the peroxide value of the cheese samples. For soft cheeses stored at 4 °C for 28 days, the peroxide value showed a significant increase, rising from an initial range of 0.56–0.79 meq/kg on day 0 to a final range of 1.73–1.96 meq/kg at the end of the storage period.

Similarly, for semi-hard cheeses stored for 60 days, the peroxide value increased progressively from an initial range of 0.41–0.63 meq/kg to a final range of 1.58–1.81 meq/kg. Hard cheeses, also stored for 60 days, exhibited a comparable upward trend, with values rising from 0.44–0.61 meq/kg on day 0 to 1.63–1.76 meq/kg by day 60.

Statistical analysis revealed significant effects of both cheese type and country of origin on the peroxide value throughout the storage duration. These results align with those of Siddique and Park [44], who reported a significant increase ($p < 0.05$) in peroxide values in goat milk Cheddar cheese over four months compared to fresh samples, indicating progressive lipid oxidation during extended storage. The consistent increase observed from day 0 to the final measurement across all cheese types in the present study further confirms this progressive oxidative trend.

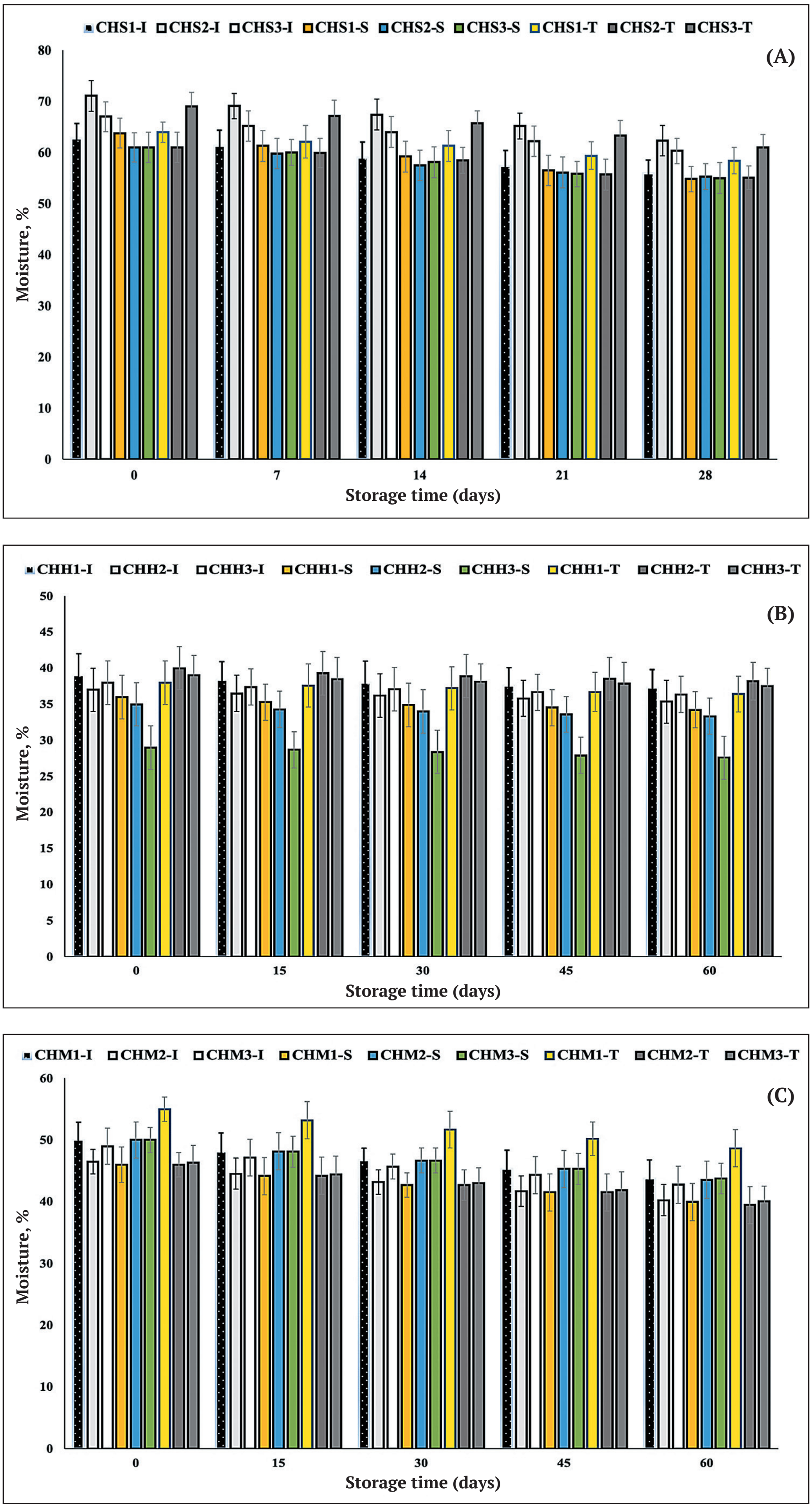

Figure 2. **Effect of storage duration on the moisture content of the cheese samples (A) Soft cheeses stored at 4 °C for 28 days; (B) semi-hard cheeses, and (C) hard cheeses stored at 4 °C for 60 days**

Рисунок 2. **Влияние продолжительности хранения на содержание влаги в образцах сыра (A) Мягкие сыры (4 °C, 28 суток); (B) полутвердые сыры и (C) твердые сыры (4 °C, 60 суток)**

Table 4. **Effect of storage duration on peroxide value (meq/kg)**
Таблица 4. **Влияние продолжительности хранения на пероксидное число (мэкв/кг)**

A. Soft cheeses						
Country	**Cheese code**	**Storage duration**				
		0 days	**7 days**	**14 days**	**21 days**	**28 days**
Country A	CHM1-I	0.61 ± 0.02	0.89 ± 0.03	1.23 ± 0.01	1.53 ± 0.02	1.79 ± 0.03
	CHM2-I	0.63 ± 0.02	0.92 ± 0.01	1.25 ± 0.01	1.55 ± 0.02	1.81 ± 0.04
	CHM3-I	0.47 ± 0.01	0.76 ± 0.01	1.09 ± 0.01	1.39 ± 0.02	1.65 ± 0.03
Country B	CHM1-S	0.58 ± 0.02	0.89 ± 0.01	1.20 ± 0.01	1.50 ± 0.01	1.74 ± 0.04
	CHM2-S	0.61 ± 0.02	0.90 ± 0.01	1.23 ± 0.01	1.53 ± 0.01	1.77 ± 0.02
	CHM3-S	0.61 ± 0.02	0.89 ± 0.01	1.23 ± 0.01	1.53 ± 0.01	1.76 ± 0.04
Country C	CHM1-T	0.51 ± 0.02	0.80 ± 0.01	1.13 ± 0.01	1.43 ± 0.01	1.68 ± 0.03
	CHM2-T	0.41 ± 0.02	0.72 ± 0.01	1.03 ± 0.02	1.33 ± 0.01	1.58 ± 0.04
	CHM3-T	0.50 ± 0.03	0.79 ± 0.01	1.12 ± 0.03	1.42 ± 0.02	1.67 ± 0.03
L.S.D. for cheese type		0.035	0.020	0.016	0.016	0.040
L.S.D. for country of origin		0.023	0.027	0.030	0.030	0.028
B. Semi-hard cheeses						
Country	**Cheese code**	**Storage duration**				
		0 days	**15 days**	**30 days**	**45 days**	**60 days**
Country A	CHS1-I	0.65 ± 0.01	0.97 ± 0.01	1.28 ± 0.01	1.58 ± 0.02	1.83 ± 0.02
	CHS2-I	0.56 ± 0.01	0.85 ± 0.01	1.18 ± 0.01	1.48 ± 0.02	1.73 ± 0.03
	CHS3-I	0.59 ± 0.02	0.87 ± 0.02	1.20 ± 0.01	1.50 ± 0.02	1.75 ± 0.02
Country B	CHS1-S	0.79 ± 0.02	1.07 ± 0.02	1.41 ± 0.01	1.71 ± 0.02	1.96 ± 0.02
	CHS2-S	0.62 ± 0.01	0.92 ± 0.01	1.23 ± 0.01	1.53 ± 0.02	1.78 ± 0.03
	CHS3-S	0.68 ± 0.02	0.98 ± 0.02	1.30 ± 0.01	1.60 ± 0.02	1.85 ± 0.03
Country C	CHS1-T	0.60 ± 0.01	0.90 ± 0.01	1.22 ± 0.01	1.52 ± 0.02	1.77 ± 0.02
	CHS2-T	0.57 ± 0.01	0.88 ± 0.01	1.19 ± 0.01	1.49 ± 0.02	1.74 ± 0.03
	CHS3-T	0.71 ± 0.01	1.01 ± 0.01	1.33 ± 0.01	1.63 ± 0.02	1.88 ± 0.03
L.S.D. for cheese type		0.027	0.026	0.026	0.026	0.026
L.S.D. for country of origin		0.026	0.033	0.027	0.026	0.026
C. Hard cheeses						
Country	**Cheese code**	**Storage duration**				
		0 days	**15 days**	**30 days**	**45 days**	**60 days**
Country A	CHH1-I	0.61 ± 0.02	0.89 ±0.02	1.23 ±0.01	1.53 ± 0.03	1.76 ± 0.04
	CHH2-I	0.57 ± 0.02	0.87 ±0.02	1.19 ±0.02	1.49 ± 0.03	1.72 ± 0.03
	CHH3-I	0.44 ± 0.02	0.72 ±0.02	1.06 ±0.03	1.36 ± 0.02	1.63 ± 0.04
Country B	CHH1-S	0.57 ± 0.02	0.89 ±0.02	1.19 ±0.02	1.49 ± 0.02	1.73 ± 0.03
	CHH2-S	0.55 ± 0.03	0.83 ±0.03	1.17 ±0.01	1.47 ± 0.02	1.72 ± 0.03
	CHH3-S	0.52 ± 0.02	0.84 ±0.03	1.14 ±0.01	1.44 ± 0.04	1.69 ± 0.03
Country C	CHH1-T	0.53 ± 0.02	0.85 ±0.02	1.15 ±0.01	1.45 ± 0.02	1.69 ± 0.03
	CHH2-T	0.56 ± 0.02	0.86 ±0.02	1.18 ±0.01	1.48 ± 0.03	1.73 ± 0.04
	CHH3-T	0.55 ± 0.02	0.85 ±0.02	1.17 ±0.02	1.47 ± 0.04	1.72 ± 0.03
L.S.D. for cheese type		0.055	0.047	0.056	0.055	0.057
L.S.D. for country of origin		NS	0.025	NS	NS	NS

* Results as mean ± SD (standard deviation); L.S.D.: Least Significant Difference; NS: Not significant ($p > 0.05$).

4. Conclusion

The present study provides a comprehensive comparison of cheese quality parameters across products from three different countries (Iran, Saudi Arabia, and Turkey). Findings reveal that cheese type significantly influences physicochemical profiles, with hard cheeses exhibiting superior antioxidant capacity due to advanced proteolysis and texture-dependent composition, while country of origin affects initial microbial quality and specific compositional variations. HMF accumulation was more pronounced in semi-hard and hard cheeses, reflecting the impact of prolonged ripening periods and the progression of Maillard reactions during storage rather than initial heat treatment. While bacterial counts were generally within acceptable limits, some soft cheese samples exhibited elevated E. coli counts, suggesting lapses in hygiene or post-processing contamination that require targeted intervention. The antioxidant potential, assessed through DPPH and reducing power assays, was highest in hard cheeses, supporting their functional food value. These findings underscore the need for strict adherence to type-specific storage protocols and enhanced monitoring of microbial safety to ensure consistent quality, particularly for imported products. Moreover, the role of cheese as a source of antioxidants may provide functional food benefits if appropriately processed and stored. Further studies should explore peptide profiling and metabolomic changes during storage to optimize cheese quality and safety.

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AUTHOR INFORMATION	СВЕДЕНИЯ ОБ АВТОРАХ
Affiliation	**Принадлежность к организации**
Mohammed A. Falih, MSc Student, Department of of Dairy Science and Technology, College of Food Sciences, University of AL-Qasim Green Al-Qasim, 51013, Iraq E-mail: mrmr26102018@gmail.com ORCID: https://orcid.org/0009-0000-6763-4815	**Фалих М. А.** — магистрант, Факультет науки и технологии молока, Колледж пищевых наук, Университет Аль-Касим Грин Аль-Касим, 51013, Ирак E-mail: mrmr26102018@gmail.com ORCID: https://orcid.org/0009-0000-6763-4815
Qausar Hamed Alkaisy, Assistant Professor, Department of Dairy Science and Technology, College of Food Sciences, University of AL-Qasim Green Al-Qasim, 51013, Iraq E-mail: qayssarhamad@fosci.uoqasim.edu.iq ORCID: https://orcid.org/0000-0002-0363-6886	**Алкаиси К. Х.** — доцент, Факультет науки и технологии молока, Колледж пищевых наук, Университет Аль-Касим Грин Аль-Касим, 51013, Ирак E-mail: qayssarhamad@fosci.uoqasim.edu.iq ORCID: https://orcid.org/0000-0002-0363-6886
Ammar B. Altemimi, Professor, Department of Food Science, College of Agriculture, University of Basrah Basrah, 61004, Iraq College of Medicine, University of Warith Al-Anbiyaa Karbala, 56001, Iraq E-mail: ammar.ramddan@uobasrah.edu.iq ORCID: https://orcid.org/0000-0001-7750-5988 * corresponding author	**Алтемими А. Б.** — профессор, кафедра пищевых наук, сельскохозяйственный колледж, Университет Басры Басра, 61004, Ирак Медицинский колледж, Университет Варит Аль-Анбия Кербела, 56001, Ирак E-mail: ammar.ramddan@uobasrah.edu.iq ORCID: https://orcid.org/0000-0001-7750-5988 * автор для контактов
Tarek G. Abedelmaksoud, Associate Professor, Department of Food Science, Faculty of Agriculture, Cairo University 1 Gamaa Street, 12613, Giza, Egypt E-mail: tareekgamal_88@agr.cu.edu.eg ORCID: https://orcid.org/0000-0002-7012-6667 * corresponding author	**Абедельмаксуд Т. Г.** — адъюнкт-профессор, кафедра пищевых наук, сельскохозяйственный факультет, Каирский университет 12613, Египет, Гиза, ул. Гамаа, 1 E-mail: tareekgamal_88@agr.cu.edu.eg ORCID: https://orcid.org/0000-0002-7012-6667 * автор для контактов

Contribution	**Критерии авторства**
Authors equally relevant to the writing of the manuscript, and equally responsible for plagiarism.	Авторы в равных долях имеют отношение к написанию рукописи и одинаково несут ответственность за плагиат.
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