Hindawi
Journal of Food Quality
Volume 2023, Article ID 6653063, 11 pages
https://doi.org/10.1155/2023/6653063

Research Article

Effect of Kinnow (*Citrus nobilis* × *C. deliciosa*) Peel Oil Coating on the Shelf Stability and Antioxidant Potential of Cheddar Cheese

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Received 18 May 2023; Revised 15 August 2023; Accepted 31 August 2023; Published 30 October 2023

Academic Editor: Rafik Balti

Kinnow peel oil has been reported to possess antimicrobial and antioxidant activities due to presence of many bioactive compounds. Therefore, the experiments reported in this research paper aimed to investigate the shelf stability and antioxidant potential of Cheddar cheese coated with Kinnow peel oil at various concentrations (0.2%, 0.4%, and 0.6%). The physicochemical characteristics, microbial counts, antioxidant potential, and sensory acceptability of Cheddar cheese were evaluated during ripening (up to 9 months, 4°C). The titratable acidity (%), pH, moisture (%), fat (%), protein contents (%), and soluble nitrogen (SN, %) of Cheddar cheese investigated in the present study showed significant ($p < 0.05$) variations among treatments during ripening. The maximum plate counts (8.36 Log cfu·g^{-1}) were observed in control cheese (T_0) ripened for 3 months whereas T_3 (9 months ripened) showed the minimum counts (5.04 Log cfu·g^{-1}) among all the treatments. The maximum (3.76 Log cfu·g^{-1}) yeast and mold counts were observed in T_0 cheese ripened for 9 month whereas the respective samples of T_3 showed the lowest values (2.89 Log cfu·g^{-1}) among all the treatments. The surfaces of Cheddar cheese coated with 0.6% Kinnow peel (T_3) did not show any visible signs of yeast and molds. The maximum (366.56 mg GAE/100 g) total phenolic contents were observed in T_3 ripened for 6 months. The maximum values (1995.02 mM TE/100 g) of DPPH radical scavenging activity were observed in T_3 ripened for 6 months. Similarly, T_3 obtained the highest sensory scores among all the treatments. Hence, it was concluded that Kinnow peel oil significantly improved antioxidant potential, sensory perception, and shelf stability of Cheddar cheese.

1. Introduction

Cheddar is a hard cheese variety having protected designation of origin (PDO) and was originated in the middle of the nineteenth century after the name of a village in Somerset, England [1]. During Cheddar cheese manufacturing, cheddaring is the process of piling and repiling of curd into heaps to prevent a decrease in temperature during cheese manufacturing. This process also squeezed out any pocket of gas (due to the activity of coliforms and clostridia) during manufacturing and is helpful in developing peculiar texture of Cheddar cheese [1]. Defined cultures comprising of

Lactococcus lactis subsp. *lactis* and *L. lactis* subsp. *cremoris*, are used for the manufacturing of Cheddar cheese.

Chemical preservatives such as potassium sorbate and sodium benzoate have been employed for the extension of shelf life of cheeses [2]. Some preservatives (nisin and natamycin) are also used in the inner side of packaging material in order to extend the shelf stability of cheeses. These chemical preservatives have been reported to cause many health hazards, like for instance, sodium benzoate may cause skin allergies as well as cancer development whereas potassium sorbate may show mutagenecity and DNA damaging activity [3]. Therefore, in order to avoid the deleterious effects of those chemical preservatives, natural preservatives should be employed for the extension of shelf life of cheeses [3]. In this regard, edible films and coatings are produced from food grade additives or edible biopolymers. Many biopolymers such as proteins (caseins, whey proteins, and wheat gluten), carbohydrates (starch, modified cellulose, carrageenan, and chitosan), lipids (sunflower oil, waxes, and Tween 20) have been reported to be used for extension of shelf life of cheese [4].

Edible films and coatings are fine edible membranes that can facilitate transfers of moisture, oxygen, and fluids [5]. Based on their raw material composition, edible coatings and films can be categorized into three basic categories: polysaccharide, essential oil, and protein-based films [6]. Essential oils (EOs) are aromatic molecules found in surplus amounts in oil sacs or oil glands at various depths in the fruit peel, primarily the flavedo and cuticle regions [7]. Citrus peels are the wastage of citrus fruit processing industry and have been reported to be rich in essential oils [8]. Citrus fruit peels are also known to contain some antioxidants; flavonoids such as hesperidine, nari-rutin, naringin, and eriocitrin, and also polyphenols such as caffeic acid, *p*-coumaric acid, ferulic acid, and sinapinic acid [9–11]. Citrus peel or its extract has been employed in pharmaceuticals, cosmetics, and food as packaging material and food industries as preservatives [8, 12]. Many studies also reported various compounds in citrus peel essential oil, e.g., D-limo-nene, β-myrcene, α-pinene, β-pinene, γ-terpinene, α-ter-pinolene, α-caryophyllene, copaene, β-phellandrene, etc. [9, 11, 13]. Moreover, these oils are mixtures of many volatile components especially monoterpene hydrocarbons [14]. The United States Food and Drug Administration classified EOs as GRAS (generally recognized as safe). These have been found to be inhibitory equally in direct oil and vapor form against a series of both Gram-positive and Gram-negative bacteria [15]. Many studies have reported the antifungal activities of citrus essential oils [14, 16, 17]. Moreover, these oils have also been considered to have bioactivity against spoilage causing microorganisms [18]. In particular, Kinnow (*Citrus nobilis* × *C. deliciosa*) peel essential oils are affordable, environmentally sustainable, and natural alternatives to sodium nitrites, nitrates, benzoates, butylated hydroxyl anisole (BHA), and other synthetic antioxidants that are frequently used in food preservation [19]. Kinnow peel oil has been found to be effective against many pathogenic and fungal strains [20, 21]. Essential oils or plant extracts containing phenolics and terpenes may be used to preserve cheese quality parameters such as texture, color, flavor, and pH [3]. In addition, higher moisture contents of cheese make it more vulnerable to mould attack, oxidation, and development of off-flavor [22]. Therefore, application of Kinnow peel oil on the surfaces of cheese would be helpful in minimizing microbial attacks and hence may extend the shelf life of such products.

To date and to our knowledge, no study has been carried out previously regarding monitoring the shelf stability and antioxidant potential of Cheddar cheese by the use of Kinnow peel oil as natural antimicrobial coating. Thus, the objective of the present study was to explore the effect of Kinnow peel oil on physicochemical characteristics, microbiological quality, sensorial acceptability, and antioxidant potential of Cheddar cheese during ripening.

2. Materials and Methods

2.1. Chemicals and Apparatus. DPPH (2, 2-diphenyl-1-picrylhydrazyl), Trolox, gallic acid, Folin–Ciocalteu reagent, and many other chemicals/standards were purchased from Merck (KGaA, Darmstadt, Germany). Analytical chemicals (sodium citrate, Sodium hydroxide, isoamyl alcohol, concentrated sulphuric acid, copper sulphate, potassium sulphate, and iron sulphate) were purchased from Lab-Scan (Dublin, Ireland). The cheese vat and other apparatus were thoroughly washed and cleaned, and manufacturing was done under hygiene condition in the laboratory. All the glassware used were properly washed and sterilized.

2.2. Procurement of Raw Materials for Development of Cheese. Fresh buffalo milk (15 L for each treatment) was procured from a dairy farm in Sargodha (Pakistan) for the production of cheddar cheese. The milk was standardized to 2.50% fat contents. Kinnow peel oil was kindly donated by the Citrus Research Institute, Sargodha.

2.3. Treatment Plan and Manufacturing of Cheddar Cheese. Cheese was manufactured by slightly modifying the method described by Hanlon et al. [23]. The milk (15 L) was pasteurized (30 min at 63°C) and cooled to 32°C with continuous slow stirring. The direct-vat-set (DVS) culture (R-704) was added (after making active in sterile skimmed milk) into the milk. Then, $CaCl_2$ (0.05% w/w) was added into the milk to accelerate the coagulation process. The rennet (Chy-Max) (3.5 mL/15 L) was added into the milk until the pH reached to 6.5. The milk was left unstirred (~45 min) until the milk was converted into coagulum. The coagulum was cut into small cubes with the help of cutting wires. The temperature of coagulum was increased stepwise until 39°C, and cooking/scalding was done for 30 min. The whey was drained at pH 6.2 to 6.3 and then cheddaring was carried out until the pH reached to 5.4. After addition of salt (1.5%) into the milled cheese matrix, it was transferred to molds and pressed (2 bar pressure with hydraulic press) for 10 h at room temperature. The cheese block was coated with essential oil according to the treatment plan (Table 1).

Table 1: Treatment plan of cheddar cheese coated with kinnow peel oil.

Treatments	Concentration of kinnow peel oil
T_0 (control)	Cheese without coating
T_1	Cheese coated with 0.2% kinnow peel oil
T_2	Cheese coated with 0.4% kinnow peel oil
T_3	Cheese coated with 0.6% kinnow peel oil

The cheese block from each treatment was cut into four identical pieces, and each piece was properly packed in polyethylene bags to minimize moisture loss and contamination. One piece was transferred into freezer immediately whereas the other 3 pieces were transferred to cold storage (4°C) room at a dairy factory and ripened for different periods, i.e. 3, 6, and 9 months. The cold storage room had clean shelves for placing the cheese samples, and its temperature was maintained at the industry. Three batches were prepared for each treatment.

2.4. Physicochemical Analysis of Cheddar Cheese. Cheddar cheese (from all treatments) was analyzed for various physicochemical parameters. The acidity (% lactic acid), moisture, and ash were investigated by following the methods described in AOAC [21]. The fat contents of cheddar cheese were determined by Gerber method. The pH of Cheddar cheese was monitored using a pH meter after calibrating with buffer of pH 4.0 and pH 7.0. The electrode was placed in the grated cheese (25 g) with a few drops of water [25]. Total nitrogen (TN, %) was estimated according to the IDF standard 20B [26]. Further, protein contents (%) were calculated by multiplying TN (%) with 6.38. Sample preparation for the determination of TN (%) and soluble nitrogen (SN, %) by Kjeldahl apparatus was done by the following manner: About 12.50 g grated cheese was taken in a blender and 50 mL of tri-sodium citrate solution (0.5 M, 50°C) was added along with 100 mL of distilled water (50°C) and blended until proper mixing. The blended sample of cheese was then poured into the beaker which was then placed in a water bath (50°C) for 60 min for further homogenization of the blended mixture. The volume of the above mixture was made up to 250 mL in a flask. About 50 mL of the above mixture was transferred into a 100 mL volumetric flask and made the volume up to the mark by distilled water. From that diluted solution, 1 mL was taken in digestion tube for the determination of TN (%) of sample. For the determination of soluble nitrogen (SN%), the left over solution (i.e. 200 mL) from the above mixture was mixed with more or less 50 mL of 0.5 HCl until pH reached 4.6. The filtrate was obtained after filtration of the mixture and 2 mL was taken in digestion flask for the determination of SN (%).

2.5. Microbiological Analysis. The cheese was analyzed for total plate counts (TPC, Log cfu/g of cheese) and yeast, and mold (Y & M, Log cfu/g of cheese) during storage period by following method as described by Broadbent et al. [27]. Firstly, grating of cheese samples from each treatment were carried out after thawing. Ten grams of grated cheese samples from each treatment was homogenized in 90 mL of sterilized tri-sodium citrate (2%, pH 7.5) water. One mL of different dilutions of the above suspension (up to 10^{-4}) was then plated on plate count agar media (Acumedia, LAB, Neogen® Culture Media, USA). The TPC were enumerated after incubating the Petri plates for 2 days at 37°C. Similarly, the counts of Y & M were also done after 2 days at 30°C by employing potato dextrose agar (Biolife, Milano, Italia).

2.6. Water Soluble Extracts (WSEs) of Cheddar Cheese. Gupta et al.'s [28] method was adopted for the preparation of WSEs of Cheddar cheese prepared in the present study. Initially, 40 g of grated cheese was thoroughly mixed (in a blender) with 120 mL of distilled water and then stirred for 2 h at room temperature. The pH was adjusted to 4.6 by adding 1 M HCl, and the final volume of the mixture was made up to 200 g by adding distilled water. Then, the sample was centrifuged at 5000 rpm for 10 min. The supernatant was filtered using Whatman No. 1 filter paper. The aliquots (in Eppendorf tubes) of WSEs of Cheddar cheese were stored at −20°C until analyzed.

2.7. Determination of Total Phenolic (TP) Contents of Cheddar Cheese. The TP contents of cheese were evaluated by following the procedure described by Reis et al. [29]. 1 mL of WSE of cheese sample was combined with 1 mL of Folin–Ciocalteu reagent (10%). After 6 min, 2 mL of sodium carbonate solution (20%) was added to the mixture. The absorbance was measured after 60 min of incubation at room temperature, at 760 nm using a spectrophotometer (Halo DB-20, UV-Vis double beam, Dynamica Scientific Ltd., Livingston, UK). The results were expressed as mg GAE/100 g of cheese.

2.8. Determination of Total Antioxidant Capacity (TAC) of Cheddar Cheese. The TAC of cheese was investigated by adopting and modifying the procedure of Prieto et al. [30]. A 0.4 mL of WSE of cheese was mixed with 4 mL of ammonium molybdate reagent (4 mM/L ammonium molybdate + 28 mM sodium hydroxide + 0.6 M sulphuric acid in 250 mL). A spectrophotometer (Halo DB-20, UV-Vis double beam, Dynamica Scientific Ltd., Livingston, UK) was used to measure the absorbance at 695 nm, following 95 min of incubation of above mixture at 90°C. Using Trolox as a standard, the TAC of cheese was computed as mg/100 g of cheese.

2.9. Determination of DPPH Radical Scavenging Activity of Cheddar Cheese. The capability of WSE of cheese to scavenge 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was measured by modifying the procedure described by Yi et al. [31]. 1 mL of WSE of cheese sample was mixed with 2 mL of DPPH solution (60 μM in ethanol). The sample was then incubated in the dark for 30 min. The absorbance was measured using a spectrophotometer (Halo DB-20, UV-Vis

double beam, Dynamica Scientific Ltd., Livingston, UK) at 517 nm. Trolox was used for the calculation of DPPH radical scavenging activity, and the results were calculated as mM Trolox equivalent (TE)/100 g of cheese.

2.10. Determination of Ferric Reducing Antioxidant Power (FRAP) of Cheddar Cheese. The FRAP of WSE of Cheddar cheese was measured by adopting and slightly modifying the procedure elaborated by Qureshi et al. [32]. 1 mL of WSE of cheese was mixed with 500 μL each of potassium ferricyanide ($K_3Fe(CN_6)$) and sodium phosphate buffer (Na_3PO_4). Then, 500 μL of trichloroacetic acid (10%) was added into the above mixture after incubating the mixture at 50°C for 20 min. To get a clear supernatant, the mixture was centrifuged (Hermle Labortechnik GmbH Siemensstr-25 D-78564 Wehingen, Germany) at 5000 rpm at 4°C for 5 min. The supernatant was then mixed with 150 μL of (0.2%) ferric chloride ($FeCl_3$), and the absorbance was taken at 700 nm using a spectrophotometer (Halo DB-20, UV-Vis double beam, Dynamica Scientific Ltd., Livingston, UK). Using Trolox as a standard, the FRAP of cheese was computed as mg Trolox equivalent (TE)/100 g of cheese.

2.11. Sensory Evaluation. The 9-point Hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 = like extremely) was used to evaluate the sensorial attributes, i.e. appearance and color, flavor, texture, as well as overall acceptability [33]. A panel of twenty trained assessors was chosen including faculty and students from the Institute of Food Science and Nutrition, University of Sargodha, Pakistan. In addition, the necessary Institutional Review Board (IRB) approval for sensory evaluation was pursued under IRB No. SU/IFSN/IRB/002.

2.12. Statistical Analysis. Using Statistix 8.1 software (Tallahassee, Florida, USA), the data were statistically analyzed. To compare the means, two-way analysis of variance (ANOVA) was used. Statistical significance was determined using a probability threshold of $p < 0.05$. The data were presented as means ± SD.

3. Results and Discussion

3.1. Physicochemical Characteristics of Cheddar Cheese. The titratable acidity (%), pH, moisture (%), fat (%), protein contents (%), and SN/TN (%) of Cheddar cheese investigated in the present study showed significant ($p < 0.05$) variations among treatment during ripening while ash contents showed nonsignificant behavior ($P \geq 0.05$) during ripening (Table 2). All freshly prepared cheeses (treatments) showed lower contents of acidity. Among the treatments, T_0 exhibited the maximum acidity (1.82%) after 9 months of ripening while T_3 showed the least (1.05%) value after 9 months of ripening. The acidity of cheese was gradually increased during ripening. On the other hand, the pH of cheese gradually increased during subsequent ripening periods. The pH values were more or less same as observed in all freshly prepared cheeses (5.30–5.36) which reached to the values in the range (5.62–5.69) at the end of ripening period (9 months). Most of the lactose is lost in whey (98%) whereas remaining amount of lactose may be expected in the cheese matrix [34]. The starter lactic acid bacteria (SLAB) as well as nonstarter LAB (NSLAB) of cheese usually convert residual lactose of cheese matrix into lactate and other organic acids in the start of ripening [34], thereby causing an increase in acidity (decrease in pH). Buffering capacity of cheese matrix may also affect the pH of cheese [35]. In addition, free fatty acids (FFAs) also contribute to the acidity of cheese during cheese ripening [36]. Mamo et al. [36] also observed similar results. On the other hand, the decrease in acidity (increase in pH) might be due to conversion of lactate into some flavoring compounds. Proteolysis also plays an important role in decrease in acidity (increase in pH) during ripening period by the production of NH_3 following amino acid catabolism [34]. Moreover, precipitation of calcium phosphate also contributed to the increase in pH during ripening [34]. The acidity is increased in the start of ripening of Cheddar cheese but afterwards these compounds are converted into other substances (flavor compounds) which cause decrease in acidity in cheese matrix [34]. El-Galeel and El-Zawahry [37] also observed a decrease in pH during early stages of ripening. It might be assumed that Kinnow peel oil might contribute to the increase in acidity as Kavas et al. [38] also reported the increase in acidity of cheese by the addition of clove and thyme essential oils.

The moisture contents of Cheddar cheese from all treatments prepared in the present study were more or less similar in the start, i.e. 38.04 to 38.93. The moisture contents were gradually decreased during ripening period (0–9 months). The decreasing trend of moisture contents in cheese during ripening period was also observed by Ramos-García et al. [39] and Khan et al. [39]. There was less decrease of moisture contents during ripening in the cheese having 0.6% Kinnow peel oil coatings which might be due to presence of oil layer over the surfaces preventing the loss of moisture from the surfaces. It was also observed visually that the cheese having 0.6% Kinnow peel oil coatings was more fresh compared to other cheese samples (treatments). The protein contents of all freshly prepared Cheddar cheese (control and oil-coated) were in the range 27.09 to 28.20%. The protein contents were increased after 3 months of ripening but afterwards it started to decrease until after 9 months of ripening. After 6 months of ripening, the decreasing trend of protein might be due to degradation of amino acids into volatile compounds [34]. Such conversions might be the main cause of decreasing trend of intact protein during ripening [40]. The SN/TN (%) of Cheddar cheese for all the treatments gradually increased during ripening. The SN/TN (%) in the fresh cheeses prepared in the present study was in the range 2.29–3.17 but at the end of investigated ripening period, the contents increased up to the range 21.82–24.02. The maximum value of SN/TN (%) was observed in T_3 having 0.6% Kinnow peel oil after 9 months of ripening. The ratio of SN to TN indicates the ripening index of cheese. The more SN depicted more ripening index of cheese. Proteolysis is the main process during cheese ripening which is

Table 2: Effect of kinnow peel oil on physicochemical characteristics (means ± SD) of Cheddar cheese during storage (4°C, 0–9 months).

Parameters	Months	Treatments			
		T_0	T_1	T_2	T_3
Titratable acidity	0	0.58 ± 0.03^{h}	0.45 ± 0.06^{i}	0.46 ± 0.05^{i}	0.53 ± 0.05^{h}
	3	0.82 ± 0.05^{e}	0.62 ± 0.05^{h}	0.40 ± 0.06^{j}	0.46 ± 0.05^{i}
	6	1.22 ± 0.05^{b}	0.83 ± 0.04^{e}	0.74 ± 0.05^{f}	0.68 ± 0.05^{g}
	9	1.82 ± 0.03^{a}	1.27 ± 0.04^{b}	1.10 ± 0.04^{c}	1.05 ± 0.04^{d}
pH	0	5.30 ± 0.05^{bc}	5.32 ± 0.0^{bc}	5.34 ± 0.05^{b}	5.36 ± 0.05^{b}
	3	5.21 ± 0.06^{d}	5.24 ± 0.06^{cd}	5.25 ± 0.04^{c}	5.26 ± 0.07^{c}
	6	5.32 ± 0.06^{bc}	5.36 ± 0.06^{b}	5.37 ± 0.04^{b}	5.41 ± 0.07^{a}
	9	5.41 ± 0.08^{a}	5.42 ± 0.04^{a}	5.44 ± 0.08^{a}	5.45 ± 0.04^{a}
Moisture (%)	0	38.93 ± 0.06^{a}	38.28 ± 1.05^{a}	38.19 ± 0.48^{a}	38.04 ± 0.07^{a}
	3	37.30 ± 0.28^{c}	37.81 ± 0.09^{b}	37.30 ± 0.25^{c}	37.90 ± 0.26^{ab}
	6	37.58 ± 0.04^{cd}	37.36 ± 0.76^{bc}	37.06 ± 0.13^{cd}	37.33 ± 0.15^{c}
	9	36.16 ± 0.65^{e}	36.69 ± 0.14^{d}	36.50 ± 0.40^{de}	37.04 ± 0.06^{cd}
Protein (%)	0	27.89 ± 0.88^{b}	27.09 ± 0.42^{b}	28.20 ± 0.23^{a}	28.16 ± 0.26^{a}
	3	28.52 ± 0.70^{b}	27.42 ± 0.60^{c}	28.62 ± 0.80^{b}	27.99 ± 0.54^{bc}
	6	27.11 ± 0.94^{cd}	27.01 ± 0.74^{d}	27.81 ± 0.74^{d}	27.41 ± 0.64^{cd}
	9	26.65 ± 0.03^{de}	26.42 ± 0.43^{f}	26.92 ± 0.63^{de}	26.52 ± 0.33^{f}
SN/TN (%)	0	2.29 ± 0.28^{j}	2.83 ± 0.12^{ij}	3.17 ± 0.13^{i}	2.95 ± 0.22^{ij}
	3	8.05 ± 0.77^{h}	8.61 ± 0.43^{g}	9.14 ± 0.84^{f}	7.75 ± 0.42^{h}
	6	12.71 ± 0.64^{d}	12.05 ± 0.81^{e}	12.39 ± 0.95^{d}	12.10 ± 0.94^{e}
	9	15.80 ± 1.33^{b}	16.66 ± 1.21^{a}	14.69 ± 1.51^{c}	15.16 ± 1.13^{bc}
Fat (%)	0	24.01 ± 0.14^{a}	23.91 ± 0.12^{a}	23.74 ± 0.11^{a}	23.51 ± 0.11^{a}
	3	23.58 ± 0.18^{b}	23.68 ± 0.28^{b}	23.39 ± 0.13^{b}	22.96 ± 0.13^{d}
	6	23.07 ± 0.07^{c}	22.87 ± 0.17^{d}	22.23 ± 0.14^{e}	22.01 ± 0.14^{ef}
	9	22.22 ± 0.55^{e}	22.12 ± 0.15^{e}	21.82 ± 0.16^{f}	21.42 ± 0.16^{g}
Ash (%)	0	$3.79 \pm 0.68b^{c}$	$3.88 \pm 0.56^{a-c}$	$3.90 \pm 0.14^{a-c}$	$3.92 \pm 0.19^{a-c}$
	3	$3.73 \pm 0.03b^{c}$	$3.91 \pm 0.03^{a-c}$	$3.88 \pm 0.09^{a-c}$	$3.84 \pm 0.01^{a-c}$
	6	4.01 ± 0.01^{ab}	$3.90 \pm 0.1^{a-c}$	$3.92 \pm 0.01^{a-c}$	3.76 ± 0.25^{bc}
	9	4.07 ± 0.04^{a}	$3.91 \pm 0.01^{a-c}$	$3.86 \pm 0.10^{a-c}$	3.72 ± 0.03^{c}

T_0 = control cheese without coating; T_1 = cheese coated with 0.2% kinnow peel oil; T_2 = cheese coated with 0.4% kinnow peel oil; T_3 = cheese coated with 0.6% kinnow peel oil. Mean values sharing different alphabetic letters are statistically significant ($p < 0.05$).

responsible for the conversion of proteins into larger and smaller peptides which are present in SN fraction of protein. Our results were concurrent with the findings of many previous studies [41–43] who also observed increased contents of SN during ripening of Cheddar cheese. The fat contents (%) of fresh cheeses prepared in the present study were in the range 23.51–24.01% but at the end of investigated ripening period, the contents increased up to the range 21.42–22.22%. The decreasing trend of fats (%) during ripening was observed in the present study which might be due to conversion of triglycerides (fats) of cheese into free fatty acids during the process of lipolysis [34]. The ash contents (%) of fresh cheeses prepared in the present study were in the range 3.79–3.92%. There was a slight increasing order of ash contents of Cheddar cheese during ripening as investigated in the present study. The increase in ash contents during storage might be due to increased dry matter (due to decreased moisture contents) during ripening period. The treatments with coatings of Kinnow peel oil showed nonsignificant ($p \geq 0.05$) increase in ash contents during storage.

3.2. Total Plate Counts (TPCs) and Yeast and Mold (Y & M) of Cheddar Cheese. Figure 1 shows the TPC and Y & M counts of Cheddar cheese samples prepared in the present study. There was significant ($p < 0.05$) effect of Kinnow peel oil coatings and ripening on TPC and Y & M counts of Cheddar cheese. In general, TPC and Y & M counts of Cheddar cheese increased during period of initial 3 months of ripening but afterwards counts started to decrease. Our results were concurrent with the results obtained by Broadbent et al. [27] and Stefanovic et al. [42]. The maximum plate counts (8.36 Log cfu·g^{-1}) were observed in control cheese (T_0) ripened for 3 months whereas T_3 showed the minimum counts (8.04 Log cfu·g^{-1}). The cheese matrix usually contains both starter lactic acid bacteria (SLAB) as well as nonstarter lactic acid bacteria (NSLAB) [41, 44]. The NSLAB may get entry into the cheese from unhygienic practices during cheese manufacturing as well as post contamination of cheese. The starter bacteria are intentionally added into the cheese milk during manufacturing process for the development of specified characteristics of any cheese variety. The activity of SLAB is desirable throughout the ripening period due to peculiar aroma, flavor, and texture of cheese. During ripening of cheese in the present study, control cheese showed the maximum counts which might be due to the activities of both SLAB and NSLAB. The lowest values of total counts were observed in T_3 because it may be expected that only SLAB were active throughout the ripening period but very less NSLAB were active in that period. The decreasing trend of total counts after 6 months of ripening might be due to autolysis of bacteria cells [44].

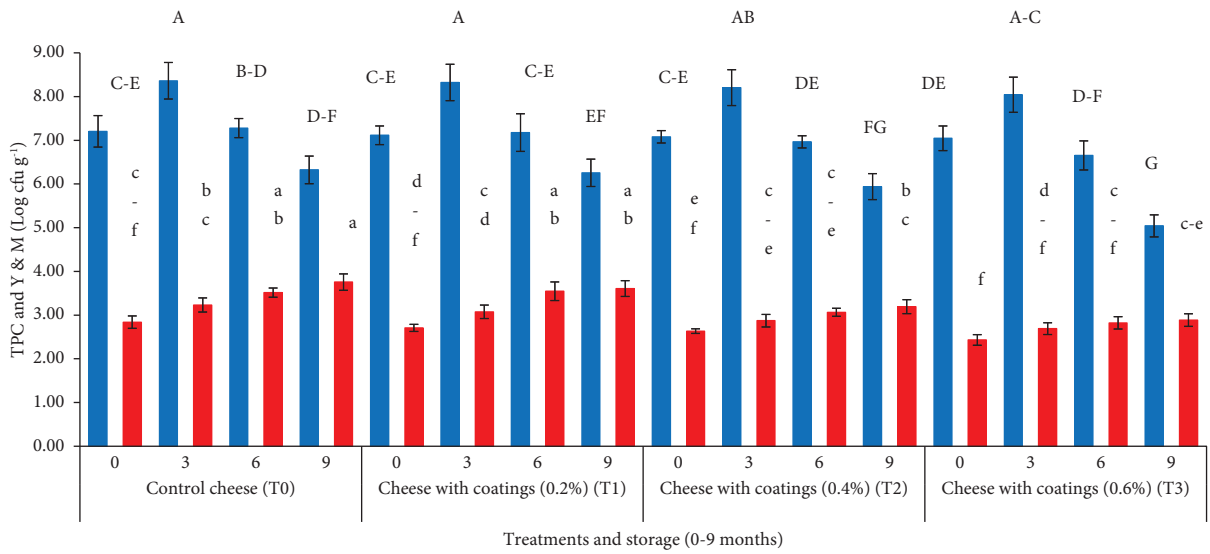

FIGURE 1: Effect of kinnow peel oil coatings (0.2, 0.4, and 0.6%) on total plate counts (TPCs) (Log cfu·g^{-1}, different capital letters showing significant ($p < 0.05$) variations, blue bars) and yeast and mould (Y & M) (Log cfu·g^{-1}, different small letters showing significant ($p < 0.05$) variations, red bars) of Cheddar cheese during storage (4°C, 0–9 months).

The Y & M counts of Cheddar cheese were gradually increased during ripening. The maximum Y & M counts (3.76 Log cfu·g^{-1}) were observed in T_0 ripened for 9 month whereas the respective samples of T_3 showed the lowest values (2.89 Log cfu·g^{-1}) among all the treatments. The growth of yeast and molds might be inhibited by increasing the quantities of Kinnow peel oil coatings. The mold growth is usually seen on the surfaces of cheese during extended period but as the cheese (T_3) surfaces were completely covered with Kinnow peel oil (0.6%), therefore mold growth was not visible at the surfaces. Many studies have reported the antifungal activities of citrus essential oils [14, 16, 46]. Moreover, these oils have also been considered to have bioactivity against spoilage-causing microorganisms [18]. Kavas et al. [38] coated Kashar cheese with thyme and clove essential oil and reported that essential oil-coated cheese had antimicrobial action as it inhibited surface growth of bacteria, yeast, and mould and extended the safety and shelf life of Kashar cheese. Librán et al. [44] observed a comparable antimicrobial effect in cheese from the aqueous extracts of various aromatic plants. Mahajan et al. [48] conducted a research on cheese fortified with pomegranate rind extract and found similar antimicrobial effects.

3.3. Total Phenolic (TP) Content of Cheddar Cheese. The findings of TP contents of WSEs of Cheddar cheese prepared in the present study are represented in Figure 2. There was significant ($p < 0.05$) effect of Kinnow peel oil coatings and ripening on the TP contents of Cheddar cheese. The TP contents of all the cheese treatments increased until 6 months of ripening period but afterwards contents started to decrease. The minimum contents (121.38 mg GAE/100 g) of TP were observed in freshly prepared control cheese (T_0). The TP contents were increased with the corresponding increase in concentration of Kinnow peel oil coatings on cheese surface. Therefore, the maximum (366.56 mg GAE/100 g) total phenolics were observed in T_3 ripened for 6 months as compared to all other treatments. The results obtained in the present study were in line with the findings of Lee et al. [49] who fortified Cheddar cheese with differing concentrations of *Inula britannica* flower extract and found that the total phenolic content increased during ripening. Moreover, the bioactive compounds present in Kinnow peel oil may diffuse into the inner matrix of paneer and become partially solubilize in water while preparing water soluble extracts of cheese. In this way, TP counts of cheese with more and more Kinnow peel oil coatings showed increasing trend of TP contents.

3.4. Total Antioxidant Capacity (TAC) of Cheddar Cheese. Figure 2 depicts the TAC of Cheddar cheese prepared in the present study. There was significant ($p < 0.05$) effect of Kinnow peel oil coatings and ripening on the TAC values of Cheddar cheese. The Cheddar cheese coated with Kinnow peel oil showed greater TAC than the control sample. The TAC of all the cheese treatments increased until 6 months of ripening period but afterwards contents started to decrease. The freshly prepared control cheese (T_0) showed the minimum TAC values (18.77 mg TE/100 g). The maximum values (23.7 mg TE/100 g) were observed in T_3. The TAC contents were increased with the corresponding increase in concentration of Kinnow peel oil coatings on cheese surface. Therefore, the maximum values (80.82 mg TE/100 g) were observed in T_3 ripened for 6 months. Our results were concurrent with the findings of Yang et al. [50] who also observed increased antioxidant activity of Cheddar cheese until 5 months of ripening but afterwards they found decreasing trend. Chen et al. [51] also reported that Cheddar cheese showed the maximum antioxidant activity until 20 weeks of ripening but after extended period, the

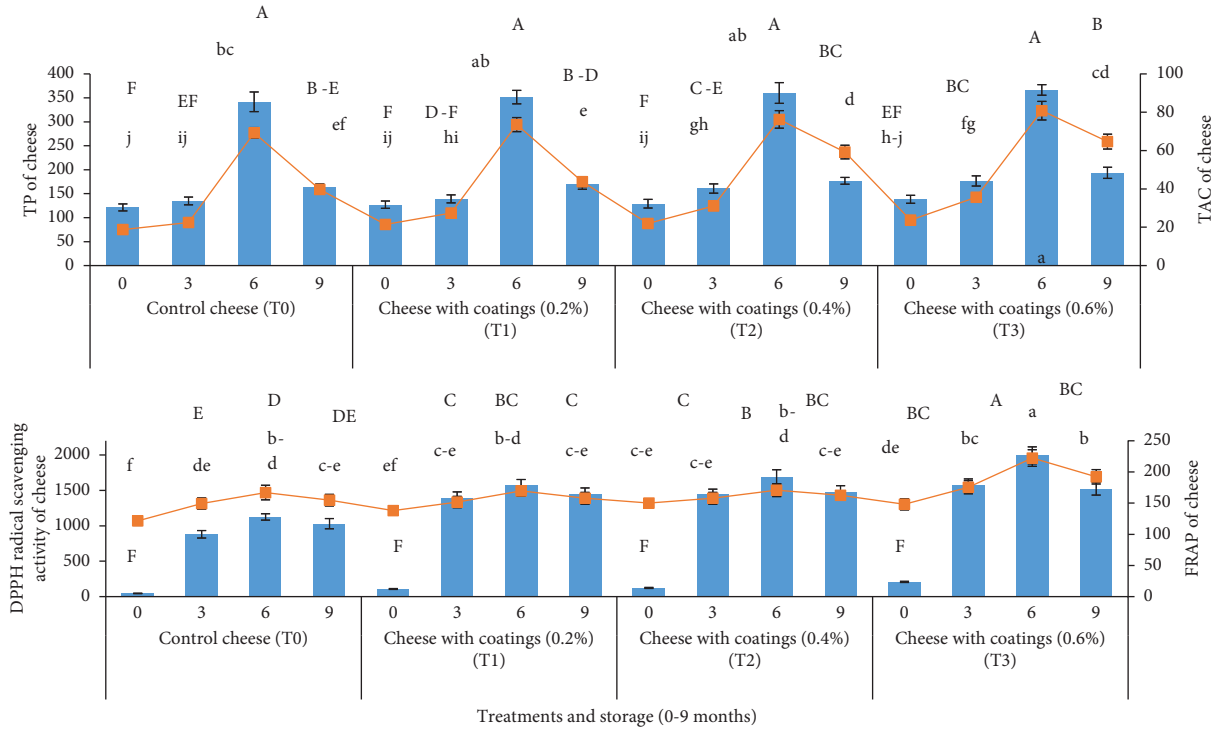

FIGURE 2: Effect of kinnow peel oil coatings (0.2, 0.4, and 0.6%) on total phenolics (TP, blue bars, mg GAE/100 g different capital letters showing significant ($p < 0.05$) variations), total antioxidant capacity (TAC, orange line, mg TE/100 g different small letters showing significant ($p < 0.05$) variations), ferric reducing antioxidant power (FRAP, orange line, mg TE/100 g different small letters showing significant ($p < 0.05$) variations), and DPPH radical scavenging activity (blue bars, mM TE/mL, different capital letters showing significant ($p < 0.05$) variations) of Cheddar cheese during storage (4°C, 0–9 months).

antioxidant activity was decreased. The antioxidant activity of Cheddar cheese might be attributed to the presence of bioactive peptides (from β-casein, α_{s1}-casein, α_{s2}-casein, and κ-casein) in the cheese matrix which are usually liberated during ripening period [50, 52, 53]. The decreased antioxidant activity after 9 months of ripening might be due to intensive proteolysis. Many peptides liberated during the extended period of ripening might have negative impact on antioxidant activity.

3.5. DPPH Radical Scavenging Activity of Cheddar Cheese. Figure 2 depicts the DPPH radical scavenging activity of Cheddar cheese prepared in the present study. There was significant ($p < 0.05$) effect of Kinnow peel oil coatings and ripening on the DPPH radical scavenging activity of Cheddar cheese. The Cheddar cheese coated with Kinnow peel oil showed greater DPPH radical scavenging activity than the control cheese. The DPPH radical scavenging activity of all the cheese treatments increased until 6 months of ripening period but afterwards contents started to decrease. The freshly prepared control cheese (T_0) showed the minimum DPPH radical scavenging activity (45.02 mM TE/100 g). The DPPH radical scavenging activity was increased with the corresponding increase in concentration of Kinnow peel oil coatings on cheese surface. Therefore, the maximum values (1995 mM TE/100 g) were observed in T_3 ripened for 6 months whereas T_0 showed the minimum values (1125 mM TE/100 g) at the respective ripening period. Our results were concurrent with the findings of Yang et al. [50] who also observed increased DPPH radical scavenging activity of Cheddar cheese until 5 months of ripening but afterwards they found decreasing trend. Chen et al. [51] also reported that Cheddar cheese showed the maximum DPPH radical scavenging activity until 20 weeks of ripening but after extended period DPPH radical scavenging activity was decreased. The DPPH radical scavenging activity of Cheddar cheese might be attributed to the presence of bioactive peptides (from β-casein, α_{s1}-casein, α_{s2}-casein, and κ-casein) in the cheese matrix which are usually liberated during ripening period [50, 52, 53]. The decreased radical scavenging activity after 9 months of ripening might be due to intensive proteolysis. Many peptides liberated during the extended period of ripening might have negative impact on radical scavenging activity. Lee et al. [49] fortified Cheddar cheese with variable concentrations of *Inula britannica* flower extract and also observed that the DPPH radical scavenging activities increased during ripening which are consistent to the findings of present study.

3.6. FRAP of Cheddar Cheese. Figure 2 presents the FRAP of Cheddar cheese prepared in the present study. There was significant ($p < 0.05$) effect of Kinnow peel oil coatings and ripening on the FRAP of Cheddar cheese. The Cheddar cheese coated with Kinnow peel oil showed greater FRAP values than the control cheese. The FRAP of all the cheese treatments increased until 6 months of ripening period but afterwards contents started to decrease. The freshly prepared

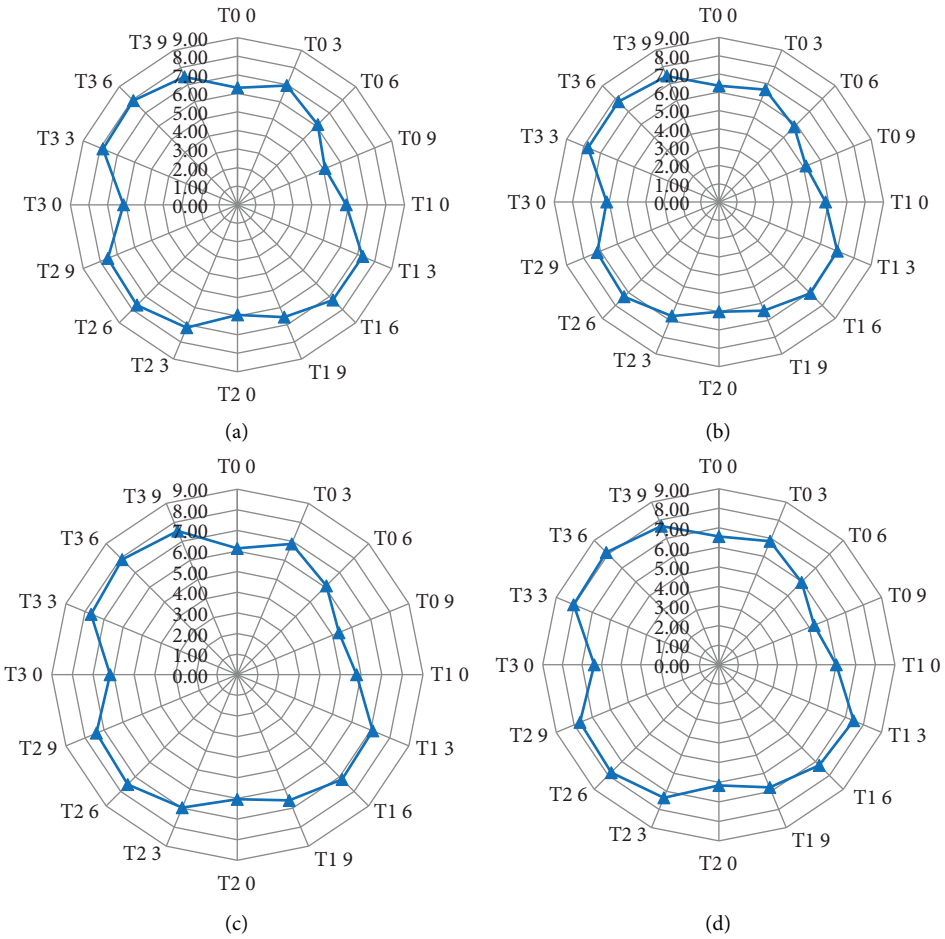

FIGURE 3: Effect of kinnow peel oil coatings (0.2, 0.4, and 0.6%) on (T_0 = control cheese, T_1 = cheese with 0.2% coating, T_2 = cheese with 0.4% coating, T_3 = cheese with 0.6% coating) sensory scores (appearance and color (a), flavor (b), texture (c), and overall acceptability (d)) of Cheddar cheese during storage (4°C, 0–9 months).

control cheese (T_0) showed the minimum FRAP values (121.48 mg TE/100 g). The FRAP values were increased with the corresponding increase in concentration of Kinnow peel oil coatings on cheese surface. Therefore, the maximum FRAP values (222.38 mg TE/100 g) were observed in T_3 ripened for 6 months. Chen et al. [51] also reported that Cheddar cheese showed the maximum FRAP values until 20 weeks of ripening but after extended period FRAP was decreased. Lee et al. [49] fortified Cheddar cheese with variable concentrations of *Inula britannica* flower extract and articulated that fortified cheese possessed the ability to contribute a hydrogen atom or a solitary electron to the reduction reaction. Similarly, Rashidinejad et al. [54] fortified cheese with (+)-catechin extracted from green tea and it was noticed that the ferric reducing antioxidant capacity of cheese increased significantly ($p < 0.05$) during storage. The reducing power of Cheddar cheese might be attributed to the presence of bioactive peptides (from β-casein, α_{s1}-casein, α_{s2}-casein, and κ-casein) in the cheese matrix which are usually liberated during ripening period [50, 52, 53]. The decreased ferric reducing power after 9 months of ripening might be due to intensive proteolysis. Many peptides liberated during the extended period of ripening might have negative impact on FRAP.

3.7. Sensory Evaluation of Cheddar Cheese. Figures 3(a)–3(d) present the scores of sensory evaluation of Cheddar cheese prepared in the present study. It was observed that Kinnow peel oil coatings had significant effect on the scores of different sensory attributes, i.e., appearance and color, flavor, texture, and overall acceptability. The minimum scores for all sensory attributes were obtained by the freshly prepared as well as ripened control cheese for all the sensory attributes. The treatment having 0.6% Kinnow peel oil (T_3) (6 months ripened) showed the highest scores for appearance and color (7.94), flavor (7.76), texture (7.91), and overall acceptability (8.13) among all the treatments. The control cheese after 9 months of ripening was not liked by the assessors. Even though, control Cheddar cheese showed

its characteristic aroma but the cheese coated with varying concentrations of Kinnow peel oil resulted in peculiar flavor. The flavor and texture of cheese are also attributed to many metabolic and biochemical processes during cheese ripening. These processes include glycolysis, proteolysis, and lipolysis which result in the conversions of many compounds into other substances [55]. The residual activities of chymosin (rennet) and casein degradation during ripening also contribute to the flavor and texture of cheese [1]. The amino acid degradation during proteolysis as well as lipolysis during ripening process are responsible for the production of different flavoring compounds which ultimately contribute the cheese flavor [34]. The intensity of flavor was increased with the increase in concentration of Kinnow peel oil. The texture of cheese with Kinnow peel oil coatings was also liked by the sensory assessors the most. The oil penetrates into the inner matrix of cheese during ripening period. Therefore, overall acceptability of cheese having coatings was also scored high. The overall acceptance of a product depicts the quality of a product. The cheese having intensive proteolysis or ripened for a longer period of time usually contain bitter tasting peptides. Such flavor was masked due to presence of Kinnow peel oil coatings. That bitter taste was observed in 9 months ripened control cheese.

4. Conclusion

On the basis of our findings, it was concluded that citrus essential oil delayed the microbial spoilage of Cheddar cheese and contributed its better sensorial properties. In addition, addition of citrus (Kinnow) peel oil also improved the antioxidant potential of Cheddar cheese.

On the basis of findings in the present study, it is suggested that the dairy industry may also use Kinnow peel oil as natural antimicrobial agent on the surfaces of cheese so as to enhance its shelf life as well as flavor.

Data Availability

The dataset supporting the conclusions of this article is included in the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors would like to thank the Research Supporting Project Number (RSPD-2023R668), King Saud University, Riyadh, Saudi Arabia.

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