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Green Extraction Methods for Flavonoid Extraction from Pomegranate Peel and its Incorporation to Cheese

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ABSTRACT

Fruit peel is a valuable source of antioxidants, such as polyphenols, flavonoids, and carotenoids. Pomegranate, which belongs to the *Punicaceae* family and is one of the oldest edible plants, contains bioactive compounds in its peels. Pomegranate peel is particularly rich in flavonoids and antioxidants. Various methods can be employed to extract antioxidants from fruit peels, including solvent extraction, supercritical fluid extraction, and ultrasound-assisted extraction. Among these methods, solvent extraction is the most commonly used method. In recent years, edible coatings have been utilized to extend the shelf life of cheeses. Flavonoids offer numerous medicinal benefits including anticancer, antioxidant, anti-inflammatory, and antiviral properties. In addition, they have been found to have neuroprotective and cardioprotective effects. The objective of this study was to extract flavonoids from pomegranate peels using suitable extraction methods. Furthermore, we developed and applied an antimicrobial coating made up of pomegranate peel onto freshly prepared cheddar cheese and explored the Physicochemical and Sensory Properties and Shelf Life of Antimicrobial Coated Cheese during Storage. This research involved the production of cheese containing varying concentrations of pomegranate peel-derived flavonoids. The antimicrobial activity of these cheese samples was assessed through microbiological analysis, including enumeration of common spoilage and pathogenic bacteria.

INTRODUCTION

Food waste is a significant global challenge, with wide-ranging environmental, economic, and social implications. One area where substantial food waste occurs is the discarded peels of fruits and vegetables. This study aimed to explore the issue of food waste, specifically focusing on the potential value and utilization of peels (Schanes *et al.,* 2016). Peels can be transformed into delicious and nutritious ingredients for various culinary applications. (Luo *et al.,* 2021). By highlighting innovative approaches and opportunities, we can reduce waste and maximize the benefits of these often underutilized resources. Food waste is a significant global challenge with wide-ranging environmental, economic, and social implications (Mourad, 2016). For example, extraction of bioactive compounds from citrus peels can yield valuable antioxidants, essential oils, and dietary fibers. Similarly, fruit and vegetable peels can be utilized in the production of animal feed, bioplastics, and compost, thereby offering sustainable alternatives to conventional methods (Sak, K. 2014).

Pomegranate (*Punica granatum* L.) has a long history (Kumari et al., 2012) and has been produced in places such as West Asia, the Mediterranean, and other parts of the world. Human research has provided positive results, indicating that pomegranate may act as a preventative agent against a variety of ailments. These investigations have primarily focused on employing pomegranate as an antioxidant and antimicrobial agent in various food items (Johanningsmeier & Harris, 2010; Kumar et al., 2021; Mohammed et al., 2024) and its therapeutic potential in conditions such as cardiovascular diseases, cancer, diabetes, and inflammatory ailments. Moreover, recent laboratory investigations, including in vitro and in vivo animal studies, have revealed a promising role of pomegranate in addressing various illnesses (Zhao et al., 2013). These results indicated that pomegranate contains bioactive substances that can play a role in both disease prevention and treatment (Fischer et al., 2011). Pomegranates are widely consumed, and their peels are typically discarded; harnessing the peel's value can aid in waste reduction and promote a circular economy (Akhtar et al., 2012). Pomegranate peel is a promising natural supplement for cancer prevention and treatment (Ismail *et al*., 2014; Aldulaimi, 2024).

Pomegranate peel extracts have been examined as potential sources of natural antioxidants for food preservation as substitutes for synthetic additives. These extracts have the potential to prolong the shelf life of food products while delivering health benefits (Lansky et al., 2007). Pomegranate peel has the potential to offer environmental sustainability advantages. In addition to its therapeutic attributes, pomegranate peel has been used in various industries (Heber, 2011). Cheese is a fermented dairy product produced from animal milk, and is renowned for its nutritional value and probiotic potential. Lactic acid production plays a vital role in the cheese-making process, typically achieved through the controlled activity of Lactic Acid Bacteria (LAB). This acidification of milk is a fundamental step in the production of nearly all cheese types, and can be achieved using either a whey culture or the inherent LAB present in the milk (George *et al*., 2018). Cheese is created by coagulating milk protein casein, and is available in a wide range of flavors, textures, and shapes (Huppertz & Chia, 2021). This study involved extracting flavonoids from pomegranate peel using suitable extraction methods, and the production of cheese containing varying concentrations of pomegranate peel derived flavonoids later on the antimicrobial activity of these cheese samples was assessed.

MATERIAL AND METHODS

This research will be conducted at the Department of Food Science, Government College University, Faisalabad. This study was conducted in three steps:

- Manufacturing of cheese coating
- Product development
- Study of antimicrobial activity of cheese coating

All phases of the present study were conducted at the Post-Graduate Research Laboratories of the Government College University Faisalabad. For product development, pomegranate peel powder and whey were acquired from a nearby market. Coating materials, reagents, and standards were purchased from recognized chemical stores. In this study, raw materials such as pomegranate peel, whey powder, milk, and culture (LAB) for the development of Cheddar Cheese were directly purchased from local vendors of the highest quality. The chemicals or reagents required in the present

concentrated effort were purchased from a scientific store in Faisalabad. The collected raw materials were transferred to the laboratory faculty of the Food Science Department, Government College University, Faisalabad.

PLANT GUIDELINES

The collection of plant material for the total flavonoid content and antioxidant analysis was conducted in accordance with institutional, national, and international guidelines and legislation. Specifically, the plant materials were sourced from a certified supplier, ensuring compliance with all relevant legal and ethical standards. The procedures for handling and analyzing the plant samples were carried out following the guidelines of the World Health Organization (WHO) and the Convention on Biological Diversity (CBD). All experimental protocols were approved by the institutional committee, ensuring adherence to the highest standards of research integrity and environmental protection.

PROCUREMENT OF RAW MATERIAL

Fully mature and ripened pomegranate fruits were purchased from the local fruit market in Faisalabad in the early hours and taken to the Department of Food Science and Technology. The collected raw material was examined for foreign contaminants such as dirt or other particles. The pomegranates were washed with distilled water and separated from pulp. The peels were then dried in a controlled environment in hot air. The dried peel was ground using a conventional mixer (MX-GX1575; Panasonic, Japan). The dried peel powder was packed into airtight PVS bags until further use and analysis.

EXTRACTION OF FLAVONOIDS FROM POMEGRANATE PEEL

The pomegranate peel was prepared from the 50 g of dried pomegranate peel powder, weighed, and mixed with 450 ml of acidified ethanol solution. The Extraction was performed under the method of ultrasonication for 30 min at 40°C, followed by centrifugation at 6000x g for 15 min at 4°C. The extraction was repeated thrice, and collected supernatants were concentrated in dryer under reduced pressure at 40°C.

TOTAL FLAVONOIDS CONTENT

The total flavonoid content is a crucial quality indicator for determining the antioxidant activity of a sample. The level of TFC in the extracts was determined spectrophotometrically according to Djeridane et al. (2006). A compound flavonoid, aluminum, with maximal absorbance at 430 nm, was formed using this technique. Rutin was used to create a calibration curve. One milliliter of the methanolic extract and one milliliter of 2% AlCl3 methanolic solution were combined. The absorbance of the reaction mixture was measured at 430 nm wavelength after 15 min of incubation at room temperature. TFC was calculated as milligram rutin equivalents per gram of DM.

ANTIOXIDANT ACTIVITY ANALYSIS

DPPH ANALYSIS

The ability of the *Punica granatum* peel suspension (PPPS) to counteract free radicals was evaluated using a spectrophotometric method with stable DPPH. Free radical-scavenging activity was determined as the percentage of antioxidant activity (AA%) at three concentrations of PPPS (1, 5, and 10 mg/ml) prepared in distilled water. For each concentration, 1 ml was mixed with 3 ml of 0.1 mM DPPH aqueous solution, and the reaction mixtures were incubated in the dark for 30 min. The change in color from deep violet to pale yellow was measured at 517 nm using a double-beam UV/VIS Spectrophotometer (Model V530, Jasco International Co. Ltd, Tokyo, Japan) at various time intervals, including 0.5 (right after preparation), 24, 48, and 120 hours. The control consisted of 0.1 mM DPPH in water, while the blank lacked DPPH, and ascorbic acid was absent from the pure distilled water.

TOTAL PHENOLIC CONTENT

The traditional Folin-Ciocalteu assay was used to determine the TPC of the pomegranate peel samples (21). In a nutshell, the reaction solution was made by combining 1 mL of the blank methanol solution, the gallic acid standard (0, 0.2, 0.4, 0.6, 0.8, or 1.0 mL), or the sample, with 0.4 mL of the Folin-Ciocalteu reagent, 1.0 mL of the Na_2CO_3

aqueous solution (10%), and 5.6 mL of distilled water. The mixture was then incubated for 30 min in the dark. The absorbance was measured at 760 nm using a UV-5500PC spectrophotometer (Metash Instrument, Shanghai, China). Data are presented as millimoles of gallic acid equivalents (mg GAE/g) per gram of dried pomegranate peel powder.

PREPARATION OF EDIBLE COATING

The whey protein isolate (10 g) was dissolved in 100 ml of non-ionic distilled water using a magnetic stirrer and hot plate for 30 min. The mixture was then heated to 90 °C with constant stirring and allowed to cool to room temperature before use. The solution was filtered using gauze to prevent the presence of lumps or insoluble materials. Next, a standard sodium hydroxide solution was used to bring the pH to 7, and 5% glycerol was added to the previous solution and stirred for 5 min. Finally, the pomegranate added to the prepared solution.

PHYSIOCHEMICAL ANALYSIS OF COATING

THICKNESS OF FILM

A spiral micrometer (Shanghai Measuring & Cutting Tool Works, Shanghai, China) was used to measure the thickness of the films at ten random locations. Within the films, the mean standard deviation was approximately 5% of the average thickness. The films were dried at 105 °C for 24 h in triplicate before their moisture content of the films was assessed using the gravimetric technique. For the purposes of this study, "film solubility" (FS) in water was defined as the portion of a film's dry matter that dissolved after being submerged in distilled water. To determine the initial dry weight (minimal), a square film sample from each film was taken, dried at 105 °C for 24 h in a ventilation drying oven, and weighed. Immersion assays were used to measure FS in 50 mL of distilled water.

ANTIMICROBIAL ACTIVITY OF FILM

The in vitro antimicrobial activity of the prepared edible films was investigated using a disc inhibition assay following the method (Zhang *et al.,* 2019) against an *E. coli* (NCDC 134) microbial strain. For this purpose, the film samples were cut into a round shape 10 mm in diameter and sterilized. Subsequently, they were placed on an *E. coli* solid culture medium surface. Finally, the Petri plates were incubated at 37 °C for 24 h. The antimicrobial activity of the films was measured in terms of their zone of inhibition (mm).

CHEESE PREPARATION

The cheeses were created using, with a few adjustments, the methods described by RodrguezHuezo et al., in 2014. First, 5 liters of fresh milk was obtained from a neighborhood store. The kept 15 min at 72 °C of pasteurization. Citric acid was added to the milk after it had been chilled to 36°C, resulting in a pH of 5.47 0.03. Then, in the process, commercial rennet was introduced at a rate of 0.12 mL per liter, along with calcium chloride at a concentration of 0.20 grams per liter. After 30 min, the curd was segmented into 1 cm^3 pieces, and the whey was drained. The mixture was then gently agitated for another 30 min. By manually stretching the curd in hot water at 75°C to a width of 6 cm, a smooth elastic paste was formed. Subsequently, the strips were immersed in water at 20°C. A 1-gram quantity of NaCl was then incorporated, and the cheese was placed into an airtight plastic bag for storage at 4°C until it was ready for analysis.

APPLICATION OF COATINGS

The Cheese samples (250 g) were coated by brushing the coating-forming suspensions on the different cheese faces and left to dry for 1 h at room temperature (15–20 °C). This is a commonly used coating application method for small-scale processes (Costa et al., 2018). Two successive coatings were applied once the cheese surface was completely dry. Afterwards, the cheeses were vacuum packaged in polyamide/polyethylene films (thickness 70 μm) and stored at 4 °C for 21 to 28 days. Uncoated cheeses were stored under the same storage conditions as the coated ones and analyzed for comparison. Quality attributes were evaluated at the initial time and after 21–28 days of storage.

TREATMENT PLAN

MICROBIOLOGICAL ANALYSIS OF CHEESE

Viable cells were counted at intervals of 1, 15, 30, and 45 days to evaluate the microbiological changes occurring on the cheese surface following the application of the coatings. For each cheese sample, 10 g cheese was aseptically collected from the top surface and placed in a stomacher bag. To this, 1 g of sodium citrate (Merck) at a concentration of 100 mL^-1, previously sterilized, was added. The mixture was then diluted at a ratio of 1:10 (w/v) and processed in a stomacher (Masticator IUL Instruments) for 1.5 minutes at 260 rpm. Decimal dilutions were subsequently prepared using peptone water at a concentration of 0.1 gram per 100 mL^-1 and were triple-plated on the appropriate substrates. *Staphylococcus spp.* were quantified using the Baird-Parker Agar Base supplemented with egg yolk and tellurite emulsion, as originally proposed by Baird-Parker. Pseudomonas species were enumerated on Pseudomonas Agar F (PAF) and incubated aerobically at 37°C for 48 h. Enterobacteriaceae was counted on Violet Red Bile Glucose Agar (VRBGA) and Tripton Soya Agar (TSA) after being incubated at 30°C for 48 h. Yeasts and molds were identified on Rose-Bengal Chloramphenicol Agar (RBC) after incubating for five days at 25°C.

ANALYSIS OF TEXTURE PROFILE

The test was conducted with some modifications to the method outlined by Pimentel-González *et al.,* (2015). First, a CT3 texture analyzer (Brookfield, United Kingdom) equipped with a 50 kg load cell was employed to assess 2 cm cubes of cheese. The samples were subsequently compressed to 50% of their original height at a double compression rate of 10 mm/s. The instrument recorded measurements of hardness (N), elasticity (dimensionless), cohesiveness (dimensionless), and chewiness (N).

COLOR

A colorimeter (Color Tec- PCM Cole-Parameter International, Accuracy Microsensors, Inc. Pittsford, NY, US), cheese color was assessed on day 1 and at the end of the storage period. The results are expressed as L*, a*, and b*, which represent the lightness, greenness, and yellowness of the sample, respectively. On the treated cheeses that were kept at 4 °C, three readings were collected at randomly from the surface. The a* value denotes colors ranging from red (+) to green (), the b* value represents yellow (+) to blue, and the L* value indicates brightness from black (0) to white (100).

STATISTICAL ANALYSIS

All data were analyzed statistically to test the significance level using the completely randomized design (two-way ANOVA) described by Montgomery et al. (2017).

RESULTS AND DISCUSSIONS

CHARACTERIZATION OF RAW MATERIAL

Pomegranate is consumed as fresh arils or processed products, such as fresh or concentrated juice, infusions, or jams. Therefore, the lack of yield in the extraction of juice and the inedible portion (i.e., pomegranate peel) may contribute to the high levels of loss and waste caused by pomegranate fruit globally in the food supply chain. Pomegranate by-products are produced in significant quantities as waste during the earliest stages of fruit processing and during the last step of fresh pomegranate aril consumption in households. The by-products of the food industry are a major source of phytochemicals, bioactive substances, and phytochemicals, such as fiber, phenolic compounds, and antioxidants. Fruits and vegetables have diverse phytochemistries and nutritional makeups. Pomegranate by-products, on the other hand, offer added value as suppliers of bioactive chemicals, highlighting the possibility of creating fresh food additives and minimizing trash in the agri-food sector. The pomegranate peel powder used in this study is a key component of this research and its potential needs to be examined. The current study was conducted to evaluate the antimicrobial activity of pomegranate peel powder coating on cheddar cheese. Different physicochemical and sensorial evaluations were performed for different treatments. The results obtained from this analysis were subjected to statistical analyses. In this chapter, the results are presented along with a discussion.

ANALYSIS OF EDIBLE COATING

THICKNESS OF EDIBLE COATING

The features of edible coatings, such as tensile strength, elongation, and water vapor permeability, are significantly influenced by their thickness (*Rokayya 2017; Rokayya & Ebithal 2019).* The appearance of the products and their ability to act as barriers against the passage of gas and water are both strongly influenced by their thickness. The diffusion rate was decreased by thickening the edible coating at a faster pace (Qiao *et al.,*2019). The preparation techniques used, such as drying, solvent evaporation duration, relative humidity, and dish surface, directly affect the thickness of the coating material (*Siracusa* et al., *2018)*. According to Park *et al., (*2018), several chemicals, including plasticizers, antioxidants, and antibacterial compounds, were added to the matrix. The control film samples in this investigation were thinner (0.142 ± 0.05 mm) than the other evaluated samples. The F_3 (0.159 ± 0.43 mm) coating sample had the thickest layer, followed by the F_2 (0.156 ± 0.28 mm) and F_1 (0.154 ± 0.83 mm) coating samples. These findings demonstrated that interactions between the functional molecules caused the thickness of the edible coating to increase as the amount of pomegranate peel extract increased (Riaz *et al.,* 2018). The thickness of the coating was unaffected (P> 0.05) by the addition of pomegranate peel extracts. Moreover, in comparison to the covered cheese, the uncoated cheese exhibited a significant amount of mould development on its surface. The outcomes demonstrate that these coatings can be used in place of synthetic coatings (Cerqueira et al., 2009).

EFFECT OF EDIBLE COATINGS ON CHEDDAR CHEESE

The initial water content was 35.5 0.7 g water/100 g cheese, whereas their respective weight-to-weight ratios for fat, protein, and ash were 26.8 0.7, 27.1 0.5, and 4.8 0.2, respectively. These cheeses can be categorized as "medium fat hard cheeses" by the Codex standard after taking into consideration their water and fat concentrations. Images of the exterior of the uncoated and coated cheddar cheese products at the beginning of storage and 60 days later (see figure 1). The covered cheddar cheese products were noticeably brighter than the untreated products. This behavior was attributed to the smoother surface of the coated cheese samples, which increased the amount of visible light reflected when compared to the uncoated cheese surface.

ANTIMICROBIAL ACTIVITY OF EDIBLE COATING

The inherent antioxidants in pomegranate peel work as effective inhibitors to restrain and slow the development of germs (see figure 1). In the food processing sector, natural antioxidant molecules are particularly helpful in delaying and controlling food product deterioration (Rokayya et al., 2018; López-Córdoba, 2021; Vasiliauskaite et al., 2022). Displays the total number of mesophilic aerobic bacteria from samples of cheddar cheese with and without edible coatings that were stored at 4°C for 28 days. Regardless of the presence of edible coatings made of pomegranate and WPI extract, all samples revealed similar bacterial counts, both at the beginning and during storage. Bacterial growth increased with time by approximately 1.7 log cycles. Using the APHA standard, tests for Salmonella, total coliform, total fungal, and standard plate count revealed no appreciable levels of microbes in the product reported by Nazim et al. (2013).

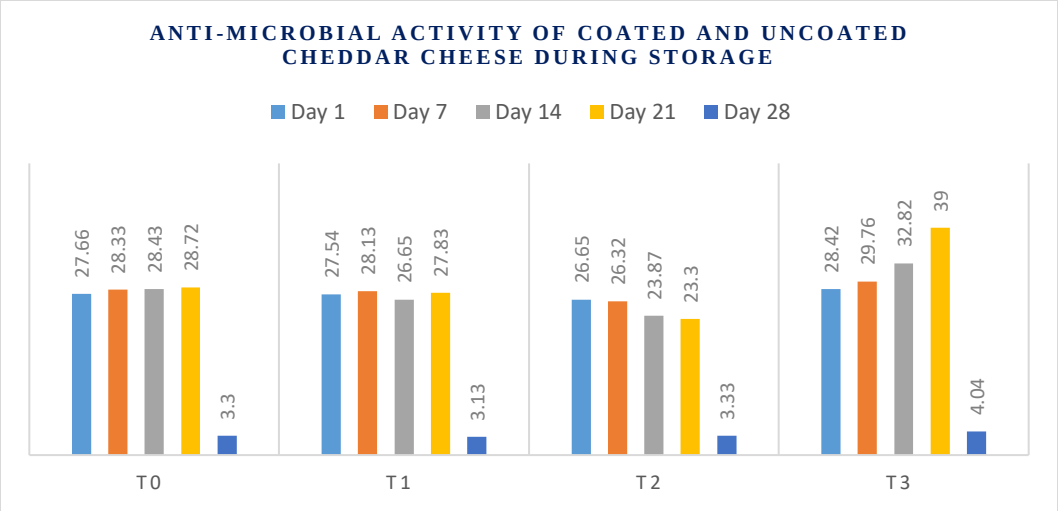

Figure 1. **Mean value for antimicrobial activity of coated and uncoated cheddar cheese during storage (x-axis shows groups and y-axis days).**

ANALYSIS OF CHEESE

PHYSIOCHEMICAL ANALYSIS OF CHEESE

The nutritional profile of a food is determined by examining its original makeup. This percentage was used to illustrate this. Proximate analysis is essential for comprehending the nutritional benefits and properties of the food we eat. Cheese supplements were evaluated for composition and the results were provided so that they could be observed. Cheese is a popular food because of its many uses, nutritional value, usability, and mouthwatering flavor. Growing knowledge of the chemistry, microbiology, and technologies involved in cheese production has led to a wide range of cheeses (Farky, 2016). Owing to its melting and stretching properties, unripe, soft, and white mozzarella cheese is ideal for preparing pizza (Nasr et al., 2015) (see figure 2).

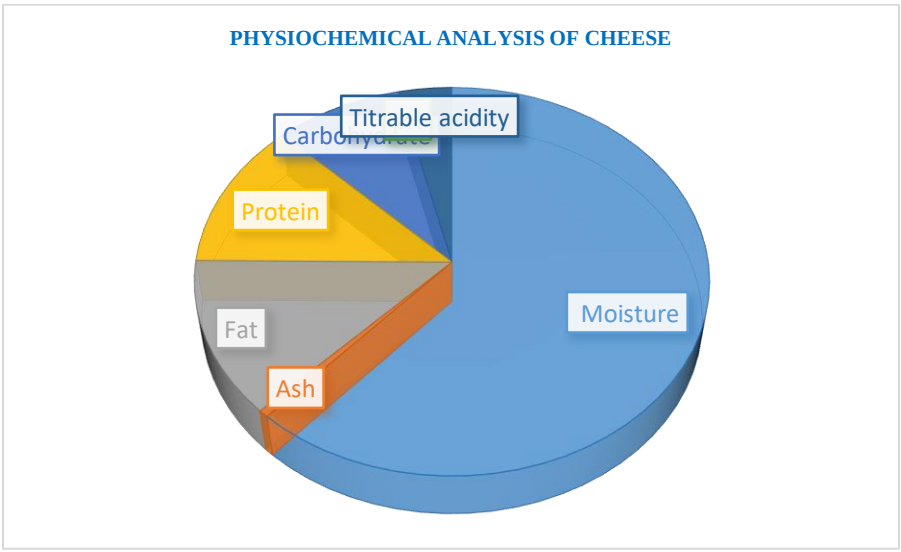

Figure. 2 **Percentage of Physiochemical Analysis of Cheese**

MOISTURE EFFECT

The moisture content of cheddar cheese plays a crucial role in its texture, flavor, and overall quality. The moisture content is an essential factor that cheese makers control during the cheese-making process. The primary roles of moisture in cheddar cheese are as follows. Texture and Consistency: High Moisture: Cheese with higher moisture

content tends to be softer, creamier, and more elastic in texture. Young Cheddar cheese with a higher moisture content is often milder and easier to slice or melt. It can be used in applications such as sandwiches and cheese sauces. Low Moisture: As Cheddar cheese ages and loses moisture through evaporation during aging, it becomes firmer, drier, and crumbly. This results in a sharper flavor and texture, which h is ideal for grating and snacking. Flavor Development: Moisture affects enzymatic and microbial activities: The presence of moisture influences the activity of enzymes and microorganisms in cheese during the aging process. This interaction is essential for the development of the characteristic flavor profile of cheddar cheese. As moisture content decreases, the flavor of cheese becomes more concentrated and complex, leading to the development of sharper and more intense flavors. Aging potential: Lower moisture content for longer aging: Cheddar cheese with lower moisture content has a longer aging potential. Extra-sharp cheddar cheeses, which are aged for several years, have very low moisture levels, allowing them to develop robust and intense flavors over time. Shelf Life: Lower Moisture for Longer Shelf Life: Cheeses with lower moisture content tend to have a longer shelf life because they are less susceptible to spoilage by microorganisms. Reduced moisture inhibits the growth of bacteria and molds, which could cause the cheese to spoil (see figure 3).

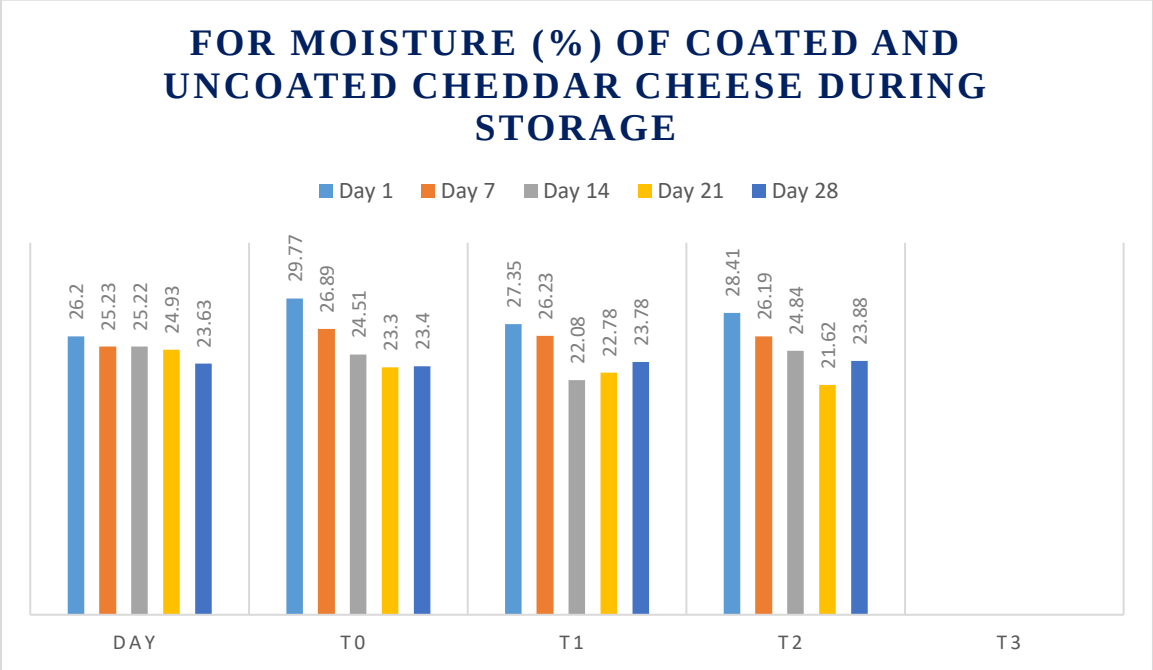

Figure 3. **Mean value for moisture (%) of coated and uncoated Cheddar cheese during storage_(x-axis shows groups and y-axis days).**

pH EFFECT

According to Cheese Perfection, pH has an impact on almost all attributes, including texture, flavor, and appearance. The pH of cheese changes and is inconsistent as it is stored. The pH of most cheeses decreases throughout the manufacturing process and the first several weeks of storage. Lactic acid is primarily responsible for the pH decline (Pastorino *et al.*, 2003). The pH of cheese samples varied between 4.1 and 4.5. The pH of the covered cheese samples was higher at the beginning of storage than that of the uncoated samples (see figure 4). The higher pH of the coating-forming solution (4.6) compared to that of cheese (4.3) was thought to be the cause of this behavior. Additionally, cheese samples that were not coated showed an increase in pH during storage, but samples that were coated had a constant pH over time. Martins *et al.*, 2016) investigated the impact of galactomannans coatings on the shelf-life extension of ricotta cheeses and observed similar behavior (see figure 4).

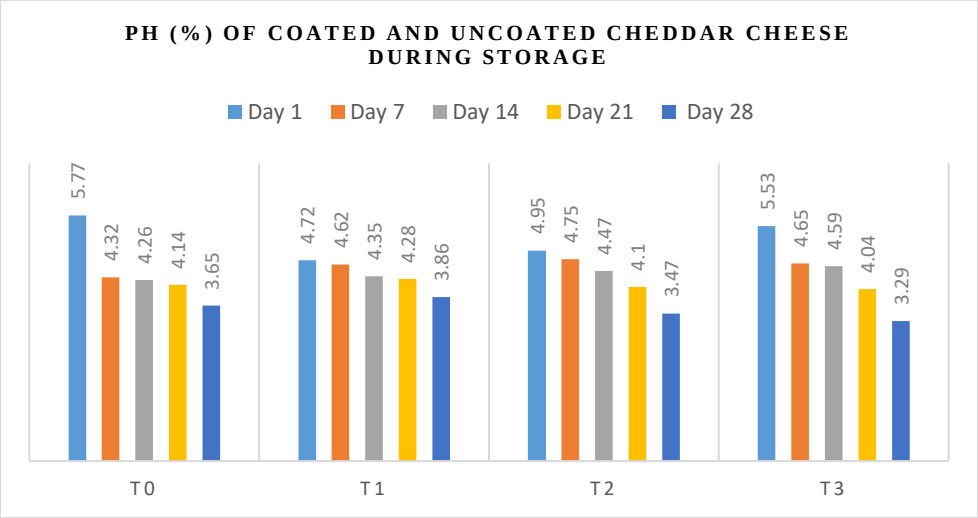

Figure 4. **Mean values of pH (%) of coated and uncoated Cheddar cheese during storage (x-axis shows groups and y-axis days)**

ACIDITY EFFECT

An important step in the production process is the controlled production of lactic acid and galactose by the same bacteria that create lactic acid (Amarita et al., 2001). When starters such as citric acid, acetic acid, and lactic acid are added to milk, the acidity of cheese is increased. Acidity gives cheese its capacity to withstand microbial degradation. Additionally, it improves the texture, flavor, and overall quality of the cheese (McSweeney, 2004). Both the treatment effects and short-term consequences of continuing to eat cheese were highly significant ($P < 0.01$). However, there was a significant difference ($P < 0.05$) between maintenance days and medication interactions (see figure 5).

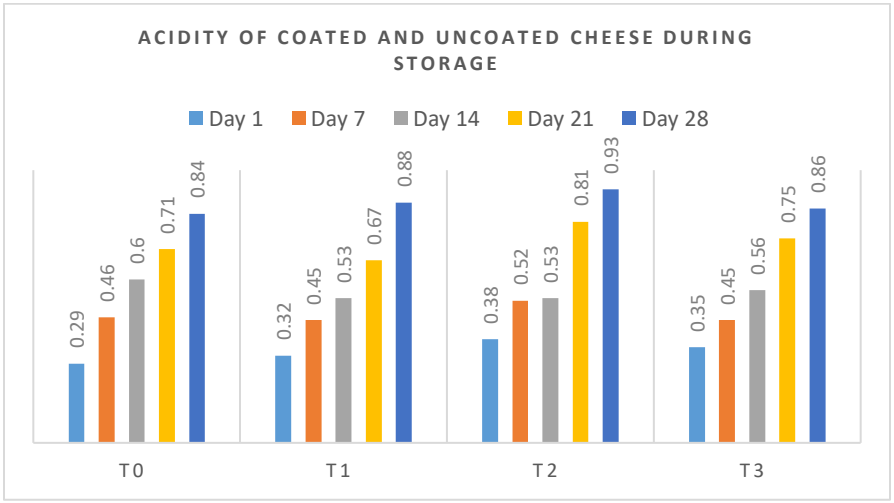

Figure 5. **Mean values of Acidity of coated and uncoated cheese during storage (x-axis shows groups and y-axis days)**

SENSORY EVALUATION

Five panelists from the Government College University Faisalabad, who were academics, employees, and research professionals, provided sensory evaluations of the cheese. According to Ahmed et al. (2019), assessors understand the sensory qualities of cheese, such as its weight, texture, appearance, flavor, and general acceptability. Taste is an essential sensory component. The assessors believed that samples with 10% extract were the most flavorful

throughout the storage period. The results from the control samples and batches containing 20% pomegranate peel powder were equivalent and did not significantly differ (p 0.05). The scores for the control samples decreased over time, although the changes were not statistically significant ($p > 0.05$). Sensory evaluation is a key tactic for product development. The acceptability of a food product's appearance, perception of its flavor, and general perception of its quality are all factors in a consumer's decision to purchase. These samples were tested 1, 7, 14, and 21, 28 days after storage. Values were generated for each of the properties that were described independently by numerical analysis performed on the combined data. The panelists were given a Performa 9-point sentimental magnitude rating for their evaluation.

TEXTURE ANALYSIS

The results demonstrated that the pomegranate peel powder extract was a substantial source of bioactive compounds with potent antioxidant effects (see figure 6). When establishing overall acceptability, it is also important to consider panelists' evaluations of the elements of color, flavor, mouth feel, and texture. Any evaluation of acceptability requires texture evaluation. It is a vital sign of the dependability and attraction of the product. This shows that a decrease in onion hardness occurs when flavonoids increase. Bolarinwa *et al.*, (2020) examined the chemical, psychological, and sensory properties of an improved pan bread. They found that the puppy-supplemented cookie had a somewhat softer texture than his superiors.

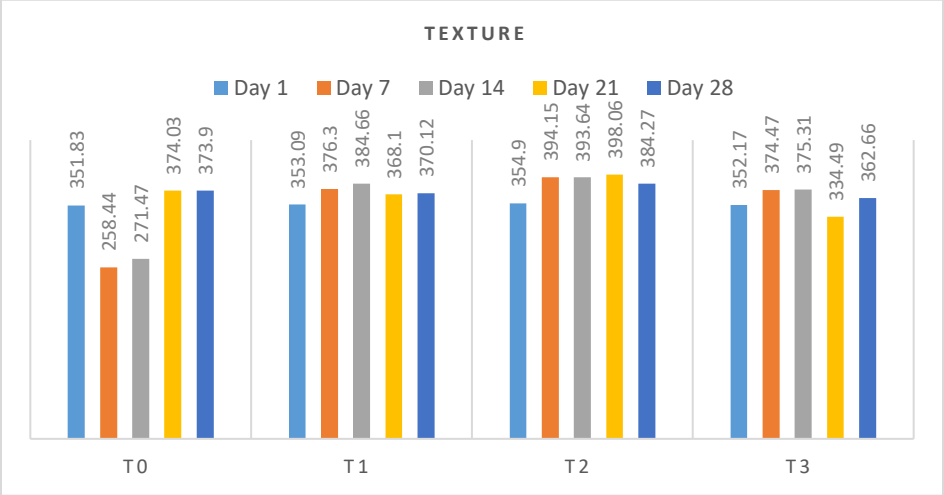

Figure 6. **Mean values of texture (x-axis shows groups and y-axis days).**

COLOR ANALYSIS

b * value

The L*, a*, and b* values of the coated and uncoated cheese samples from Lab. The color difference (E) between the uncoated and coated cheese samples was approximately 1.4 at the beginning and after 60 d of storage. A value of E = 3 has been proposed as the absolute color discrimination threshold for cheeses. This suggests that the color characteristics of the cheddar cheese were not significantly altered by the addition of edible coatings (see figure 7).

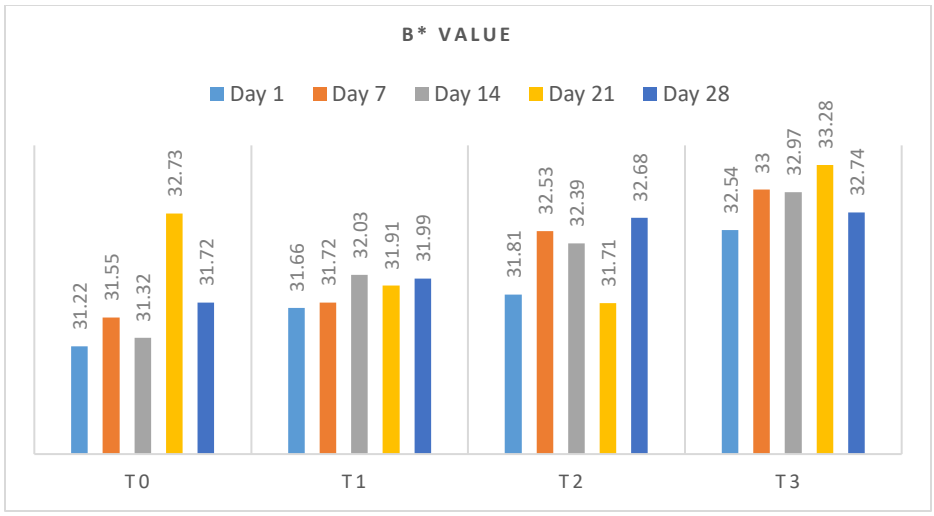

Figure 7. **Mean values for b* value (x-axis shows groups and y-axis days)**

a* VALUES

The a* value represents the red-green component of a color, with positive and negative values denoting red and green standards, respectively. The L*, a*, and b* variables can be used to translate dermatological variables. The A* value and erythema were correlated. ANOVA results for cheese coated with various amounts of pomegranate peel powder. It was demonstrated that the a* value decreased from 5.37 to 6.44 during the course of many interventions (see figure 8).

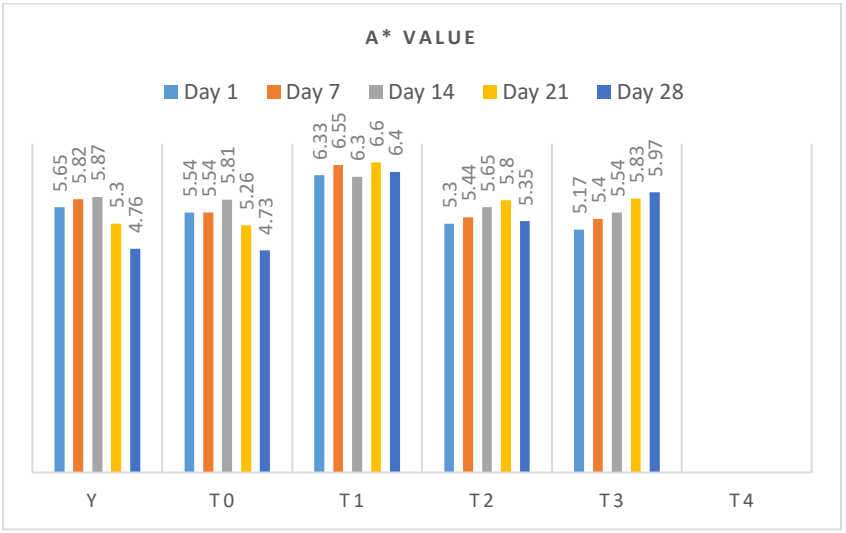

Figure: 8 **Mean values for a* value (x-axis shows groups and y-axis days)**

L* VALUE

The three dimensions of the CIELAB color system, often known as CIE L* a* b*, show a quantitative relationship between colors. The color of each item was ascertained using a tool called a colorimeter. L* generates gloomy colors at 0 and brilliant colors at 100. It has been determined that different treatments caused the L* value to vary from 50.95 to 54.44% (see figure 9) L* a* b*.

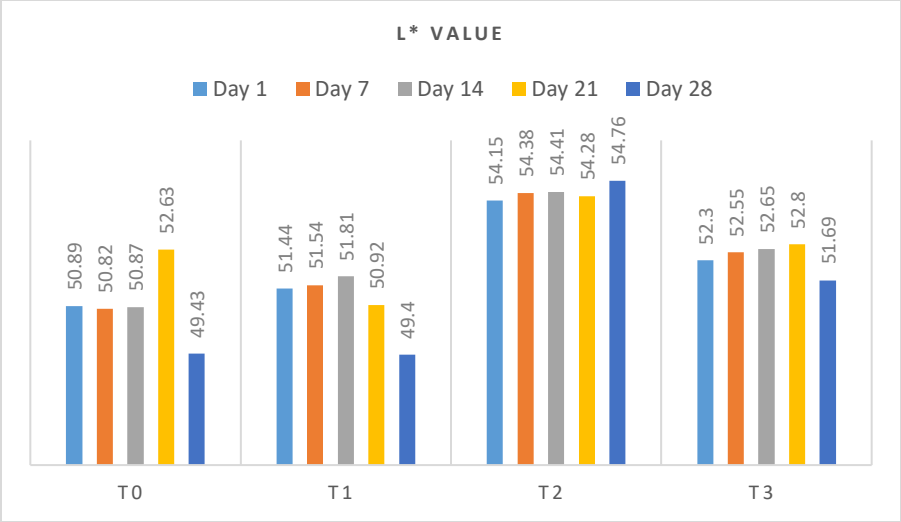

Figure 9 **Mean values for l* value (x-axis shows groups and y-axis days)**

OVERALL ACCEPTABILITY

The review and assessment of all the indicated scores, as well as the panelists' opinions regarding the characteristics of color, taste, mouth feel, and texture, are key components in determining overall acceptability. Overall acceptability rating in relation to analysis of variance for coated cheese made with different amounts of pomegranate peel powder. Statistical findings showed that the overall acceptance of coated cheese was not considerably impacted. Additionally, the general acceptability of cheese did not differ considerably among the various treatments. Definitive results for the cheese how that, across treatments, the overall acceptability score on a 9-point hedonic scale varied from 8.16 to 5.73. The overall acceptability score showed a decreasing trend in relation to the treatments, but was not significantly different among them (see figure 10).

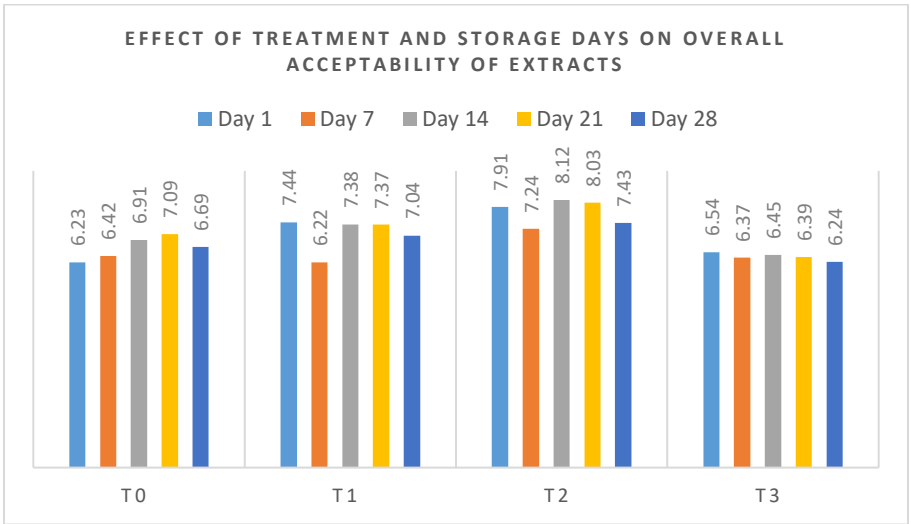

Figure 10. **Effect of treatment and storage days on overall acceptability of extracts (x-axis shows groups and y-axis days)**

CONCLUSIONS

The presence of an antimicrobial edible coating of pomegranate peel powder on fresh cheeses was evaluated to represent small-scale cheese producers that sell cheese unpacked or wrapped in paper. The applied coating significantly improved the appearance and slowed the color changes by preserving moisture in the cheese. Coating with incorporated indigenous peel extract decreased the growth of spoilage microorganisms during prolonged cheese storage, thus significantly improving the flavor of cheese. In general, the coating of pomegranate peel powder of

cheese as a base for edible coating is beneficial for both small- and large-scale manufacturers. This type of active edible coating with antimicrobial effects could be an excellent addition to both package-free and vacuum-packaged cheeses produced by manufacturers aiming for sustainability, enhanced quality, and extended shelf life of the final product.

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CONFLICT OF INTEREST
Authors declare that they have no conflicts of interest.

ETHICS STATEMENT
This study involved a sensory evaluation with human participants. Ethical approval for the sensory evaluation was obtained from the Institutional Review Board (IRB) of Government College University Faisalabad, Department of Food Science (Approval No: GC-123). All participants were provided with detailed information about the study and gave their informed consent prior to participation. The study adhered to the principles outlined in the Declaration of Helsinki, ensuring participant confidentiality and voluntary involvement. No personal identifying information was collected, and participants were free to withdraw from the study at any time without any consequences.

CONSENT TO PARTICIPATE
Corresponding and all the co-authors are willing to participate in this manuscript.

CONSENT FOR PUBLICATION
All authors are willing for publication of this manuscript.

DATA AVAILABILITY
Although adequate data have been provided in the form of tables and figures, all authors declare that if more data are required, then the data will be provided on request.

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