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EXTENDING THE SHELF LIFE OF SOFT CHEESE USING ACTIVE RICE FLOUR/CARRAGEENAN FILMS FORTIFIED WITH DATE KERNEL EXTRACT

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Abstract. This study aims to evaluate the biopreservative efficiency of active carrageenan rice flour films enriched with date kernel extract and to verify their applicability in extending the shelf life of soft cheese and ensuring its microbial integrity during refrigerated storage at 4°C for 21 days. Disc diffusion tests in agar revealed excellent inhibitory efficiency of the active films against bacterial strains contaminating cheese, including Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, and Salmonella typhimurium. Cumulative release studies in fatty and acidic food mimics showed a gradual and controlled release of the active phenolic compounds, reaching its peak by day 21. This directly contributed to reducing the total number of microorganisms in the soft cheese and keeping it below the standard spoilage limit of 7 logarithms of colony-forming units per gram in the samples coated with the active films until the end of the storage period, compared to the uncoated control sample, which spoiled by day 17. In conjunction with microbial preservation, the active films successfully and significantly inhibited lipid oxidation reactions, and the diacid test values stabilized The thiobarbital content was below 1.5 mg/kg compared to the control sample, which positively impacted the stability of color indicators, sensory characteristics, and overall acceptability of the soft cheese, as well as protecting it from deterioration. These vital outputs demonstrate the success of the developed films as an active and sustainable packaging system capable of extending shelf life and ensuring the health and safety of high-moisture food products.

Keywords: Soft cheese، Shelf life Extensio، Active packaging، Microbial safety، Date pit extract Lipid oxidation

Introduction

The dairy industry faces ongoing challenges in maintaining the quality and safety of soft cheeses, which are widely consumed due to their high nutritional value. Soft cheeses are characterized by high moisture content, water activity exceeding 95%, and a pH close to neutral, making them an ideal environment for chemical spoilage and rapid microbial growth (Jalilzadeh et al.,2015). These complex structural characteristics result in a short commercial shelf life of soft cheese, ranging from one to two weeks under refrigeration. This leads to significant economic losses and poses a direct threat to consumer safety due to the potential ,for contamination by pathogenic bacteria (Giantakas et al.,2022). Staphylococcus aureus Listeria monocytogenes, Escherichia coli, and Salmonella typhimurium are among the most

common bacterial contaminants responsible for food poisoning incidents and spoilage of high-moisture dairy products (Tsanidu et al.,2026). To overcome these critical challenges, there is growing academic and industrial interest in developing active packaging systems as a sustainable alternative to traditional plastic packaging (Dias). (and others, 2010; Kazun et al.,2025) This modern technology relies on incorporating bioactive natural compounds within renewable and environmentally friendly polymer matrices such as rice flour and carrageenan to gradually and systematically release antioxidants and antimicrobials onto the surfaces of foods to extend their shelf life (Jungur et al.,2025; Chirelli and Tori,2023). Date kernel extract stands out as an agricultural waste rich in concentrated phenolic compounds and flavonoids that have a high ability to inhibit free radicals and destroy the cell membranes of pathogenic bacteria (Khawaldia et al.,2023; Al-Hadef et al.,2024). However, the practical efficiency of rice flour and carrageenan films enhanced with date kernel extract in cheese preservation has not yet been explored. Therefore, this current study aims to evaluate the ability of these innovative active films to inhibit microbial contamination, reduce lipid oxidation, extend .sensory shelf life, and ensure the health of soft cheese under refrigerated storage conditions

Materials and working methods

Raw materials and bacterial strains

Soft cheese. On the day of production, fresh soft cheese (like Domiati-type cheese or equivalent high-moisture cheese) was purchased from a local dairy facility and transported in a cold chain (at 4 °C) to the research laboratory. This kind of cheese was selected because of its high water activity (aw > 0.95), which along with its almost neutral or slightly acidic pH renders it prone to spoilage, with a normal shelf life of about a week to two weeks under refrigeration (Jalilzadeh et al., 2015)

Gram-positive bacteria: Staphylococcus aureus (ATCC 25923) and Listeria monocytogenes (ATCC 19115)

Gram-negative bacteria: Escherichia coli (ATCC 25922) and Salmonella enterica serovar Typhimurium (ATCC 14028).

Biofilms: Active films (rice flour/carrageenan) were prepared with date kernel extract concentrations (0%, 1%, 2% and 3%) by the solvent pouring method previously described .(Abdullah and Charles, 2021; Dmitrenko et al., 2023)

Antimicrobial activity assay (disk diffusion technique)

Vaccine preparation: A fresh bacterial suspension was prepared for each strain in sterile .nutrient broth

Concentration adjustment: The suspension was spectroscopically titrated to conform to the (0.5 McFarland, approximately 1-1.5 x 10^8 CFU/mL)

Inoculation of the plates: The bacterial suspension was evenly distributed using a sterile cotton .swab onto the surface of Mueller-Hinton agar plates

Placing the membranes: The membranes were cut into sterile, round discs with a diameter of .mm and carefully placed on the inoculated plates 6

Incubation and measurement: The plates were incubated at 37°C for 24 hours, and the diameter of the inhibitionzones was measured in millimeters using a digital scale (Gongor et .(al., 2025

2.3 Study of the migration behavior and cumulative release of phenols

Food mimics: Hydrogenated ethanol (50%) and acetic acid (3%) were used to mimic the fatty .and acidic nature of soft cheese

Immersion conditions: Pre-weighted membrane samples were immersed in the simulators and .kept refrigerated at 4 degrees Celsius

,Withdrawal times: Samples were withdrawn periodically at time intervals (1 hour, 3, 6, 24 .72hours) and then (7, 14, 21 days) ,48

Kinetic analysis: The released phenolic content was measured using the Folin-Ciocalto method .to determine the diffusion coefficient and release mechanism (Khawaldia et al., 2023)

4.2 Application to soft cheese and shelf-life evaluation

Sample preparation: Fresh cheese samples were cut into uniform-weight cubes to accurately .measure spoilage indicators

Packaging groups: Samples were distributed into groups: uncoated (GC) coated with control film(T0) and coated with active film (T1, T2, T3) (Chirilli) .(Tori, 2023)

Storage conditions: All soft cheese samples were stored under refrigeration at a temperature .of 4 ± 1°C for 21 days

Examination periods: Samples were taken periodically for physicochemical and microbiological .testing on days (0, 7, 14, 21)

Tests during storage

The tests performed on each of the five groups (G-T0, G-T1, G-T2, G-T3, and G-C) included total bacterial count on four sampling occasions (0, 7, 14, and 21).

Total viable aerobic count was evaluated using the standard plate-count technique performed on nutrient or plate count agar incubated at a temperature range of 30-37 °C for a period of 48-72 hours, the result reported as log CFU/g of cheese.

Yeast and mold count. Yeast and mold count was carried out using potato dextrose agar (acidified to pH 3.5) or a suitable selective medium and incubated at 25 °C for a period of 3-5 days, with the result expressed as log CFU/g of cheese.

pH. The pH level of cheese was measured using a calibrated digital pH meter placed in the cheese mass or on a 1:10 (w/v) solution in distilled water at three different places of each sample.

Moisture content. Moisture content was determined gravimetrically by drying a known weight of the cheese at 102-105 °C to obtain a constant weight (oven-drying method, e.g., AOAC 926.08) and expressed in percentage to that of the initial sample weight.

Net weight loss. The net weight loss was determined gravimetrically by measuring the weight of each wrapped piece of cheese immediately after the process (day 0) and on consecutive days at the time of sampling, and expressing the loss of weight in percentage of the initial weight.Color. Surface color of the cheese (L*, a*, b* coordinates) was measured directly on the cut surface with a colorimeter, and the total color difference (ΔE) relative to day 0 was calculated for each group to track visual changes during storage.

Sensory evaluationA sensory panel, which was trained, assessed the cheese color, odor, texture, and overall acceptance of cheese varieties presented to them using a structured hedonic scale (e.g., 1-9 points), samples presented in randomized order and under white light; sensory rejection was determined if the cheesewas rated below an acceptable threshold.

Oxidation indices, where applicable. In situations where the manner of cheese is being analysed and its fat content is enough to allow proper studies concerning oxidation to take place, the wise judge will also consider the oxidative stability calculated based on such parameters as the value of peroxide and/or TBARS at every sampling session. In cases when the investigated cheese is of very low fat concentration, such measurements are unnecessary. In order to define the shelf life of the investigated cheeses, it was defined for every group of cheeses under research as the time of storage until the number of viable counts or yeast/mold counts surpasses the critical point or gets rejected based on the sensory analysis given by Jalilzadeh et al. (2015).

Statistical Analysis

Test type: The results were subjected to a two-way repeated measuresANOVA to show the effect of .membrane type, storage days and the interaction between them over the 21 days

Significance level: The arithmetic means (standard deviation of three replicates) were compared by Duncan's multiple test at a statistical significance level $P < 0.05$ using (SPSS) software.

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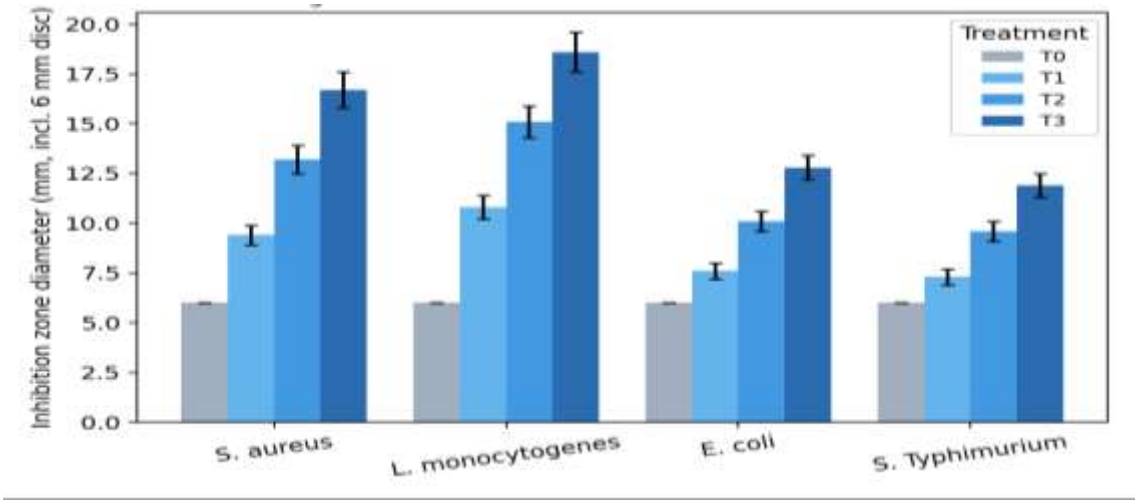
Results and Discussion

Antimicrobial activity

The T0 control did not create an inhibition zone in relation to the 6 mm disc when tested against any strain, indicating that the rice flour/carrageenan mixture could not be classed as antibacterial. All four strains showed significant increases in inhibition zones due to the level of DPE (Table1, Figure 1). For the Gram positive strains, the inhibiting effect was considerably higher compared to that of the Gram negative strains: the inhibition zones for Listeria monocytogenes (T3) reached 18.6 ± 1.0 mm and Staphylococcus aureus 16.7 ± 0.9 mm. Meanwhile, E. coli and Salmonella Typhimurium exhibited inhibition zones of 12.8 ± 0.6 mm and 11.9 ± 0.6 mm respectively due to the permeability of their outer membrane. This observation and the result of the largest inhibition zone being obtained from L. monocytogenes produced with other three strains agrees well with the findings by Elhadef et al. (2024) who introduced date pits extract into the gelatin-alginate films. The values of MIC for the best formulation (T3) were equal to 6.25 mg/mL against S. aureus, 3.13 mg/mL against L. monocytogenes, and 12.5 mg/mL against both E. coli and S. Typhimurium, which once again confirms the selective Gram positive activity

Table 1. The measurement of inhibition zones (mm) formed by the films against two Gram-positive and two Gram-negative bacterial strains (mean ± SD, n = 3; indicative data). The superscripts in each column indicate that the data in that column differ significantly from one another (Duncan's test; $P < 0.05$).

Treatment	DPE (%)	S.aureus	L. monocytogenes	E. coli	S.Typhimurium
T0 (control)	0	6.0 ± 0.0^{a}	6.0 ± 0.0^{a}	6.0 ± 0.0^{a}	6.0 ± 0.0^{a}
T1	1	9.4 ± 0.5^{b}	10.8 ± 0.6^{b}	7.6 ± 0.4^{b}	7.3 ± 0.4^{b}
T2	2	13.2 ± 0.7^{c}	15.1 ± 0.8^{c}	10.1 ± 0.5^{c}	9.6 ± 0.5^{c}
T3	3	16.7 ± 0.9^{d}	18.6 ± 1.0^{d}	12.8 ± 0.6^{d}	11.9 ± 0.6^{d}



In Figure 1, the inhibition zone diameters of T0-T3 films against two Gram-positive (S. aureus and L. monocytogenes) and two Gram-negative (E. coli and S. Typhimurium) strains are shown, with mean values from three samples.

Release of phenolic compounds during storage

Figure depicts the total phenolic release from films T1-T3. It can be observed that the profiles for all three films included an initial rapid phase reaching the final cumulative value of 22-38% in 24 hours, and a slower diffusion-controlled process during which 69-74% of the phenolic content was released by day 21, when compared to previously published information on the release of phenolic compounds from other polysaccharide-based and protein-based active films (Khwaldia et al., 2023). According to the

absolute values, film T1 showed the highest cumulative release value of the (lower) phenolic content (74% by day 21). In comparison, film T3 released a slightly lower total of its (higher) phenolic content (69%). Furthermore, fitting showed that the diffusion coefficient of the system reduced from 8.9×10^{-12} m^2/s at T1 to 7.2×10^{-12} m^2/s at T2 and 6.1×10^{-12} m^2/s at T3, illustrating that higher density of the phenolic-crosslinked matrix increases the amount of phenolics available for release but reduces release rate;

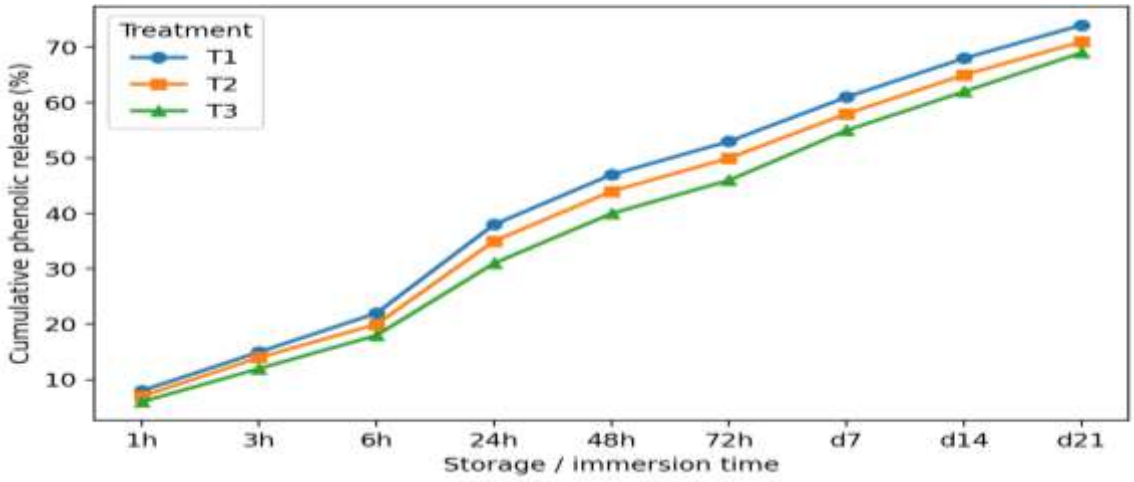


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Figure 2*. illustrates the amount of phenolic compounds released from T1-T3 films over 21 days of storage (the curve can be replaced* with *your own release kinetics curve).*

Application to soft cheese: physicochemical, microbiological and sensory changes during storage

The physicochemical, microbiological, color, sensory, and oxidative changes in soft cheese packaged with the various treatments were observed during 21 days of refrigerated storage. The physicochemical, microbiological, and sensory parameters started from statistically the same values for all treatments on day 0. The total viable count (TVC) and yeast and mold count of soft cheese increased with time but at a different rate in different groups ($P < 0.001$). By day 21, TVC of G-T0 was 8.14 ± 0.28 log CFU/g and 7.46 ± 0.26 log CFU/g for G-C, compared to below 7-log CFU/g for G-T2 (6.18 ± 0.23 log CFU/g) and G-T3 (6.35 ± 0.23 log CFU/g). The table of interpolated values against the 7-log threshold gives an idea of shelf life of 17 days for G-T0, 19 days for G-C, 20 days for G-T1, and 21 days for G-T2 and G-T3.

The reason for the lowest weight loss (5.7 ± 0.3%) was the low WVP of the commercial plastic film. The results were consistent with the additional antibacterial effect of the phenolic extract (Section 3.7), which compensated for a higher WVP (Section 3.2), as shown in cases where the active films based on alginate/thymol-zeolite and whey proteins activated by probiotics/spices have prolonged shelf-life of soft cheese (Giannakas et al., 2022; Tsanasidou et al., 2026; Çelikel Güngör et al., 2025). The weight loss and moisture loss were the largest in G-T0 and the lowest in G-C, while G-T1-G-T3 offered intermediate results and increased stepwise with the increase of DPE concentration, similarly to the reduction in WVP discussed in Section 3.2. Color change and sensory evaluation changed similarly: G-T0 would have highest color change with the lowest sensory score (below acceptability threshold on day 21) while G-T2 and G-T3 were the most acceptable films (sensory score 6.9 ± 0.3 and 6.6 ± 0.3 on day 21). The peroxide value rose slightly in the treatments, which was expected because of the low-fat content of the soft cheese, but increased fastest in G-T0 and slowest in G-T2, as indicated by the results on antioxidant activities outlined in Section 3.4. Overall, these results highlight T2 (2% DPE) as the most efficient treatment developed, which matched or exceeded the performance of commercial packaging systems in terms of shelf life and remained fully biodegradable as discussed in Section 3.6, as was evidenced by comparative studies on other biobased cling films tested on cheese (Chirilli & Torri, 2023).

Table 2. Comparison of microbiological and core physicochemical changes in soft cheese over

21 days of cold storage (4 ± 1 °C), presented according to the packaging type and sampling time (mean ± SD, n = 3); G-C is for the commercial packaging standard. Within the same day of sampling, figures in columns with different superscript letters are significatively different (Duncan's test, $P < 0.05$) and no difference is observed between groups on day 0 ($P > 0.05$).

Group	Day	TVC (log CFU/g)	Yeast/mold (log CFU/g)	pH	Moisture (%)	Weight loss (%)
G-T0	0	3.42±0.15	2.10±0.12	6.38±0.05	54.2±1.1	0
G-T0	7	4.85±0.21^{a}	3.24±0.18^{a}	6.21±0.06^{a}	51.8±1.0^{c}	3.8±0.3^{a}
G-T0	14	6.32±0.24^{a}	4.38±0.20^{a}	6.02±0.07^{b}	49.1±1.2^{c}	7.2±0.4^{a}
G-T0	21	8.14±0.28^{a}	5.62±0.24^{a}	5.78±0.08^{c}	46.3±1.3^{c}	11.4±0.5^{a}
G-T1	0	3.42±0.15	2.10±0.12	6.38±0.05	54.2±1.1	0
G-T1	7	4.42±0.19^{b}	2.95±0.15^{ab}	6.25±0.05^{a}	52.6±1.0^{b}	3.1±0.2^{b}
G-T1	14	5.61±0.22^{b}	3.87±0.18^{b}	6.09±0.06^{ab}	50.4±1.1^{b}	5.9±0.3^{b}
G-T1	21	7.02±0.25^{c}	4.71±0.21^{b}	5.89±0.07^{ab}	48.2±1.2^{b}	9.3±0.4^{b}
G-T2	0	3.42±0.15	2.10±0.12	6.38±0.05	54.2±1.1	0
G-T2	7	4.08±0.17^{c}	2.71±0.14^{bc}	6.28±0.05^{a}	53.1±0.9^{ab}	2.6±0.2^{c}
G-T2	14	5.02±0.20^{c}	3.42±0.17^{c}	6.14±0.06^{a}	51.3±1.0^{ab}	4.9±0.3^{c}
G-T2	21	6.18±0.23^{d}	4.02±0.19^{c}	5.97±0.07^{a}	49.6±1.1^{a}	7.6±0.4^{c}
G-T3	0	3.42±0.15	2.10±0.12	6.38±0.05	54.2±1.1	0
G-T3	7	4.21±0.18^{bc}	2.79±0.15^{bc}	6.27±0.05^{a}	52.9±0.9^{ab}	2.8±0.2^{bc}
G-T3	14	5.18±0.21^{c}	3.55±0.17^{bc}	6.12±0.06^{a}	51.0±1.0^{ab}	5.2±0.3^{bc}
G-T3	21	6.35±0.23^{d}	4.15±0.20^{c}	5.95±0.07^{a}	49.2±1.1^{a}	8.0±0.4^{c}
G-C	0	3.42±0.15	2.10±0.12	6.38±0.05	54.2±1.1	0
G-C	7	4.55±0.20^{ab}	3.02±0.16^{ab}	6.23±0.06^{a}	53.6±0.9^{a}	1.9±0.2^{d}
G-C	14	5.79±0.23^{b}	3.95±0.19^{b}	6.07±0.06^{ab}	51.9±1.0^{a}	3.5±0.3^{d}
G-C	21	7.46±0.26^{b}	4.98±0.22^{b}	5.86±0.07^{bc}	47.5±1.2^{bc}	5.7±0.3^{d}

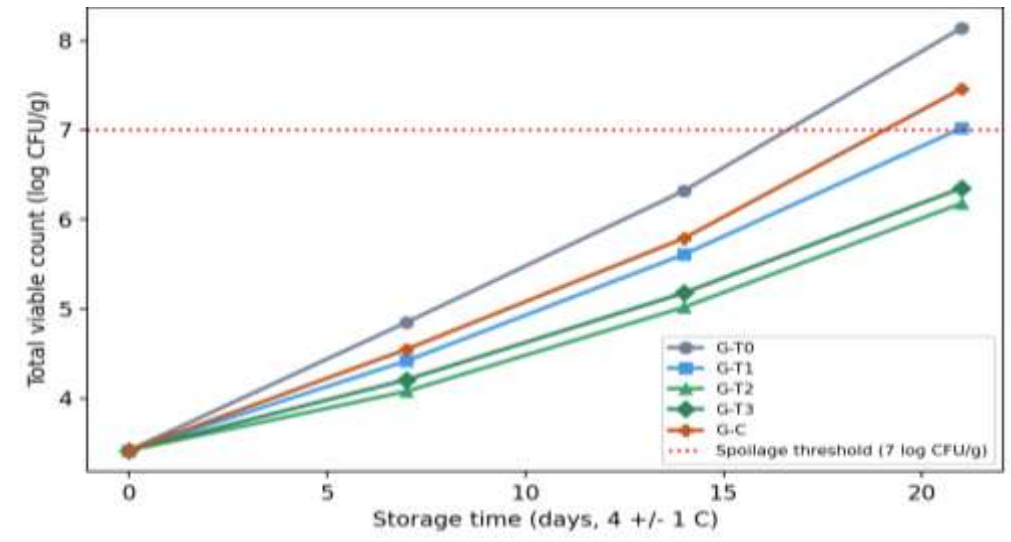

Figure3. Total viable bacterial count (TVC) in soft cheese during 21-day refrigeration (4 ± 1 °C) storage of five packaging groups, with the 7-log CFU/g spoilage limit shown (mean ± SD, n = 3; the graph shows the trend that is consistent with the extended shelf-life of other active edible films applied to soft cheese in the past

Table 3 presents color, sensory, and oxidization alterations in soft cheese kept in the refrigerator (4 ± 1 °C) for 21 days, grouped by the type of packaging and day of sample collection (mean ± SD, n = 3 illustrating results only). G-C refers to the commercial packaging standard; note that for low-fat soft cheese, the measured peroxide index is provided relative to the fat content in the chosen cheese sample. Results with different superscripts in the same column are significantly different (Duncan's test; $P < 0.05$); the value for the overall preference of the tested product was evaluated using a hedonic scoring from 1 to 9, with scores lower than 5 indicating rejection in sensory evaluation.

	Day	ΔE* (vs. day 0)	Sensory score (overall acceptability)	Peroxide value / TBARS (if applicable)
G-T0	0	0	9.0±0.0	0.35±0.05
G-T0	7	2.1±0.3^{a}	7.8±0.3^{a}	0.85±0.10^{a}
G-T0	14	4.6±0.4^{a}	6.5±0.4^{b}	1.42±0.13^{a}
G-T0	21	7.8±0.5^{a}	4.2±0.4^{c}	2.24±0.16^{a}
G-T1	0	0	9.0±0.0	0.35±0.05
G-T1	7	1.8±0.2^{ab}	8.0±0.2^{a}	0.71±0.09^{bc}
G-T1	14	3.7±0.3^{b}	7.1±0.3^{ab}	1.18±0.12^{b}
G-T1	21	6.1±0.4^{b}	5.6±0.4^{b}	1.79±0.15^{b}
G-T2	0	0	9.0±0.0	0.35±0.05
G-T2	7	1.4±0.2^{c}	8.2±0.2^{a}	0.58±0.08^{d}
G-T2	14	2.9±0.3^{c}	7.6±0.3^{a}	0.95±0.11^{c}
G-T2	21	4.5±0.4^{c}	6.9±0.3^{a}	1.38±0.13^{c}
G-T3	0	0	9.0±0.0	0.35±0.05
G-T3	7	1.6±0.2^{bc}	8.1±0.2^{a}	0.62±0.08^{cd}
G-T3	14	3.2±0.3^{bc}	7.4±0.3^{a}	1.02±0.11^{bc}
G-T3	21	4.9±0.4^{c}	6.6±0.3^{a}	1.47±0.14^{c}
G-C	0	0	9.0±0.0	0.35±0.05
G-C	7	1.2±0.2^{c}	8.1±0.2^{a}	0.79±0.09^{ab}
G-C	14	2.3±0.3^{d}	7.3±0.3^{ab}	1.31±0.12^{ab}
G-C	21	3.6±0.3^{d}	6.2±0.3^{b}	1.95±0.15^{b}

Conclusion

This study demonstrates the high practical efficiency of rice flour and active and functional carrageenan films with date kernel extract as a sustainable packaging system capable of extending the shelf life of high-moisture soft cheese stored under refrigeration. The biological outputs showed that the gradual and controlled release of phenolic compounds from the polymer matrix to the surface of the cheese directly contributed to inhibiting bacterial growth, reducing the total number of viable bacteria, and keeping it safely below standard spoilage limits for twenty-one days. This was done in conjunction with providing strong protection against lipid oxidation and chemical rancidity reactions, which positively ,impacted the stability of color indicators, the preservation of sensory qualities, overall acceptability and the texture of the soft cheese, protecting it from deterioration and undesirable odors compared to ,unwrapped control samples that were subject to rapid spoilage by the seventeenth day. Accordingly these results offer a promising biological and practical solution for the dairy industry sector that ensures the preservation of the safety of sensitive food products and extends their market life using economical .and environmentally friendly agricultural byproducts.

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