



Merging the spring onion extract into soft cheese as a rich natural phenolic ingredient to improve its antioxidant, functional, and sensory properties

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Abstract

The dairy industry has added a wide range of useful ingredients to its dairy products in response to consumers' shifting lifestyles and desire for healthier diets. Despite the extensive usage of preservatives and antioxidants, the dairy industry is coming to understand the demand for natural food items free of synthetic additives. The current study aim to determine the impact of spring onion (*Allium fistulosum*) extract on the antioxidant, functional, and sensory aspects of ultrafiltration cheese (UF-soft cheese). Spring onion extracts are incorporated into UF-soft cheese at levels of (0.1, 0.3, and 0.5%). HPLC was used to determine the total phenolic compounds and water-soluble vitamin content of spring onion extract samples, using an Agilent 1260 series HPLC. To separate phenolic chemicals, the Eclipse C18 column (4.6 mm x 250 mm ID x 5 m) was employed. Furthermore, chemicals, colors, acetaldehyde and diacetyl components, total phenol, antioxidant content, and sensory qualities were evaluated during one month of cold storage. The chlorogenic (1021.22 µg/g) and gallic acid (915.83 µg/g) levels were found to have the greatest phenolic components in spring onion ethanol extract. Moreover, the extract is very high in some vitamins, particularly vitamin C (771.15 g/g) and vitamin B6 (254.85 g/g). The chemical properties, total phenol content, antioxidant activity, and sensory scores of cheese were improved by the incorporation of spring onion extract compared to control cheese. During storage, the taste and flavor of all cheese treatments were enhanced, with T2 having the best taste and flavor, and the total sensory score is listed in descending order of acceptability for cheese: T2 > T3 > T1 > Control. Therefore, the addition of spring onion extract as a rich source of natural antioxidant agents can provide nutritional value and a unique appealing flavor profile to the cheese, making it more healthy and enjoyable for consumers.

Keywords Spring onion extract · Phenolic compounds · Vitamins · Antioxidant activity · UF-soft cheese

Introduction

Spring onions (*Allium Fistulosum*), also known as scallions or green onions, belong to the *Allium* family, which also includes garlic and onions [30]. They are known for their distinct flavor and are commonly used in various culinary dishes worldwide [31]. Spring onions are low in calories and are a good source of dietary fiber, which can support digestive health [31]. They also contain essential minerals

such as potassium, calcium, and magnesium, which are important for various bodily functions, including maintaining healthy bones and regulating blood pressure [14]. Spring onions are rich in beneficial compounds such as essential vitamins, including vitamin C, vitamin K, and B vitamins such as folate, antioxidants, and bioactive compounds [31].

Synthetic additives, such as antioxidants and preservatives, are commonly used in the food industry. However, there is an increasing demand for natural food products without synthetic additives [7, 24, 40]. Spring onion fortification in food provides a natural alternative to synthetic additives that could appeal to consumers who prefer natural food products. Spring onion is a rich source of natural antioxidants, such as phenolic compounds, that have been shown to have health-promoting properties [27]. Fortifying

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food with spring onions could increase antioxidant activity, potentially providing health benefits to consumers. These antioxidants can help neutralize harmful free radicals in the body and reduce oxidative stress, potentially reducing the risk of chronic diseases [29, 43]. Moreover, spring onions acquire natural antimicrobial properties due to the presence of sulphur compounds, such as allicin. These compounds have been shown to exhibit antimicrobial activity against various bacteria and fungi [53]. Some studies have suggested that the bioactive compounds found in spring onions may possess health-promoting properties [48]. For instance, they may have anti-inflammatory and anticancer effects [1]. Incorporating spring onion extract into food products could provide a convenient way to consume these beneficial compounds, and it may help inhibit the growth of harmful microorganisms, potentially improving the shelf life and safety of the food.

Ultrafiltration (UF) is a process used in cheese production that involves the use of a specialized filtration technique. Ultrafiltration is a membrane-based filtration process that selectively separates and concentrates components of a liquid based on their molecular size [46]. UF soft cheese may contain higher levels of protein content and bioactive peptides as well as lower lactose content compared to traditional soft cheese due to the concentration of milk proteins during the UF process. So, consuming foods like UF soft cheese may help support muscle growth, reduce the risk of chronic diseases, and are a suitable alternative for individuals who have difficulty digesting lactose. There are many recent studies that prepare cheese rich in vegetables for enhancing flavor and texture, increasing nutrient content, and providing health benefits [16, 18, 20, 21].

Spring onions have a distinct and savoury flavor that can enhance the taste of soft cheese. The addition of spring onion extract can provide a unique and appealing flavor profile to the cheese, making it more enjoyable for consumers [36]. Soft cheese enhanced with spring onion extract can be used in a variety of culinary applications. It can be enjoyed on its own, spread on bread or crackers, used as a topping for salads or sandwiches, or incorporated into various recipes. The unique flavor and potential health benefits of the cheese can add a delightful twist to a wide range of dishes. Spring onion fortified cheese could provide a unique product that differentiates itself from other types of cheese in the market. This could potentially attract consumers who are looking for novel and unique food products.

Therefore, the purpose of this study is to assess the possibility of employing spring onion extract, which is a rich source of naturally occurring antioxidant compounds, to promote consumers' health. First, measure the amount of water-soluble vitamins and total phenolic compounds in the spring onion extract using high-performance liquid

chromatography (HPLC). The chemical composition, total phenol content, antioxidant activity, color measurements, acetaldehyde and diacetyl components, and sensory characteristics of the fortified UF-soft cheese were also analyzed in order to determine the most appropriate level of fortification of spring onion extract that yields the best nutritional and sensory qualities without sacrificing the quality of the cheese.

Materials and methods

Materials

The Animal Production Research Institute and the Agriculture Research Centre provided fresh full-cream UF retentate, while a nearby market provided salt. Samples of spring onions were gathered from Egyptian local markets. Sigma Aldrich provided DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid). The source of animal rennet was Hansen's Laboratories in Denmark. The lactic acid strains used to make soft cheese came from the dairy department of the National Research Centre in Egypt. Chemicals and materials of analytical quality were all employed.

Methods

Producing powdered dehydrated spring onions

To get rid of dust and dirt particles, spring onion samples were thoroughly cleaned with tap water. The samples' outer shell was then peeled off, and the spring onion bulb and leaves were chopped into small pieces before being dried at 40 °C in a Memmert GmbH UNB 200 hot oven for 72 h. Using a grinder, the dried samples were reduced to a fine powder and then placed inside glass vials. A high-speed mixer was used to grind the dry material into a powder, which produced fine spring onion powder that had been dehydrated. The powdered spring onion was kept in dark, glass vials at 4 °C for research on application, analysis, and extraction.

Producing an extract from spring onions

After being soaked in 80% ethanol and agitated for 24 h with an orbital shaker, spring onion powder was centrifuged for 15 min at 7000 rpm. After that, the extracts were filtered via a vacuum filtering assembly, and a Heldoph G1 rotary evaporator in Germany recovered the solvents at 40 °C [23]. The extracted materials were evaluated for further tests and kept in a brown container at -20 °C.

HPLC analysis of spring onion extract

The phenolic compounds and water soluble vitamins in SE were analyzed using an Agilent 1260 series HPLC. To separate phenolic chemicals, the Eclipse C18 column (4.6 mm x 250 mm ID x 5 m) was employed. At a flow rate of 0.9 mL/min, the mobile phase was composed of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B). The following mobile phases were programmed in a linear gradient: 0 min (82% A), 0–5 min (80% A), 5–8 min (80–60% A), 8–12 min (60% A), 12–15 min (60–85% A), and 15–16 min (82% A). The ZORBAX SB-C8 (4.6 mm x 150 mm ID x 5 m) was used to separate water soluble vitamins. The mobile phase was composed of water (A) with 0.01% TFA (pH 2.9) and methanol (B) at a flow rate of 1.5 mL/min. The mobility phase was planned in the following order: 0 min (90% A), 0–1 min (90–70% A), 1–4 min (70–50% A), 4–8 min (50–90% A), and 8–10 min (90% A). For phenolic compound and B-complex vitamin solutions, the injection volumes were 10 and 5 L, respectively. At 280 nm, the multi-wavelength detector was monitored.

Manufacturing of soft cheese with spring onions

Freshly whole cream UF retentate was used to make ultrafiltration soft cheese. It was treated at 80 °C for five minutes, salted with 1.5% salt, chilled, and adjusted to 42° C [17]. Four sections of the UF-retentate were produced; the first, which was left unaltered, was used as the control. The remaining three batches were each combined with spring onion extracts (SE) at the following ratios: (0.1, 0.3, and 0.5 g)/100 g full cream UF-retentate for T1, T2, and T3, respectively. Starter cultures of 2% *S. thermophilus* CH-1 and *L. bulgaricus* Lb-12 DRI-VAC were added to each treatment for approximately two hours at 42 °C. After that, the rennet was added to each treatment at a ratio of (0.02 g for each 1.0 kg UF- milk). Then packed in 50 mL plastic cups and allowed to coagulate. Cheese samples from various treatments were refrigerated at $5 \pm 2^\circ\text{C}$ for a month and samples were examined when they were still fresh and every 10 days. All determinations were done in triplicate.

Examination of chemicals

The contents of soft cheese in terms of total solids, total nitrogen, fat, and ash were ascertained using the Association Official Analytical Chemist [4]. Multiplying the TN % by 6.38 yielded the protein content. pH-meter with microprocessor (HANNA Instruments, pH 211, Italy) was used for measuring the pH value. Water soluble nitrogen (WSN) was calculated using a modified version of the Alizadeh et al. [3] technique as an indicator of cheese proteolysis. Using this

procedure, 20 g of cheese samples were mixed for 5 min at speed setting 10 in a mixer (Heidolph No. 50 111, Type RZRI, Germany) with 100 ml of distilled water (40 °C). Whatman no. 42 filter paper was used to filter the extract, and the filtrate was then utilized to calculate WSN. $[100 - (\text{Moisture} + \text{Ash} + \text{Protein} + \text{Fibre} + \text{Fat})]$ is the percentage of carbohydrates.

Spring onion soft cheese color measures

Hunter Assoc. Lab Inc., VA, USA used a Hunter colorimeter model D2s A-2 to measure the color of the cheese samples. The appropriate button on the colorimeter was used to evaluate the color values, namely l, a, and b. The l value ranges from black (0) to brightness (100). The a value indicates the red color (positive) to the green color (negative), the b value indicates the yellow color (positive), and the negative value indicates the blue color.

Spring onion soft cheese aroma component test

Using a spectrophotometer (Shimadzu, 240-UV-Vis, Japan), the concentrations of acetaldehyde and diacetyl in soft cheese samples were determined in accordance with the Conway microdiffusion semicarbazide procedures as outlined by Lees and Jago [33]. A millilitre of a one μmol semi-carbazide solution was pipetted into the Conway microdiffusion cell's inner wall. Following a quick weighting of 3 g of soft cheese sample in the other compartment, the cell was converted, plastered, and incubated for 90 min at 37 °C. After being moved to a 10 ml volumetric flask, the inner wall solution was diluted with distilled water to reach the desired volume. A spectrophotometer (Shimadzu, 240-UV-Vis, Japan) was used to evaluate the absorption of acetaldehyde and diacetyl at 224 and 270 nm, respectively.

Cheese extracts preparation

A 100 mL quick-fit conical flask was filled with 10 g of cheese. 20 millilitres of methanol/water (80:20) were then added. Following 30 min of shaking in an ultrasonic water bath, the leftover solution was filtered into a 25 mL measuring flask and then extracted solvent was used to complete the volume to 25 mL, as reported by Mohamed et al. [38]. The final extracts were divided up and stored in glass-sealed jars for various analyses.

Antiradical properties

The scavenging activity of cheese supernatant was measured using stable DPPH radicals (DPPH) and stable ABTS radicals (ABTS) tests developed by Brand-Williams et al. [8,

45], respectively. After 30 min in the dark at room temperature (25 °C), the degree of de-colorization was determined in a spectrophotometer (Shimadzu spectrophotometer, UV-Vis. 1201, Japan) at 517 nm for the DPPH and 734 nm for the ABTS radical-scavenging activity tests. Control solutions, DPPH and ABTS solutions devoid of cheese supernatant, were made in the same way as the test combination. The following formula was used to compute ABTS and DPPH scavenging activities:

$$\text{Cheese antiradical activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

The absorbance of the control (DPPH or ABTS solution) is A_0 , while the absorbance of the sample is A_1 .

Total phenol content (TPC)

The TPC of cheese extracts was measured calorimetrically at 625 nm with the Folin-Ciocalteu reagent, as reported by Rashidinejad et al. [44]. In a 25 mL volumetric beaker, soft cheeses extract (0.5 mL), deionized water (20 mL), and 0.625 mL of the folin-ciocalteu reagent were mixed. After 3 min, 2.5 mL of saturated Na_2CO_3 (35%) solution was added. Deionized water was used to dilute the material to volume. After 1 h, the absorbance of the sample was measured at 625 nm against a blank using a Hitachi U-3210 (Hitachi, Ltd., Tokyo, Japan) double-beam ultraviolet-visible spectrophotometer. Gallic acid was used as a standard to create the calibration curve, with concentrations ranging from 2.5 to 20 $\mu\text{g}/25\text{ L}$ of test solution.

Cheese sensory assessment

Panelists are chosen based on their ability to detect and describe sensory attributes accurately. The 20 dairy science department members from the National Research Centre in Giza, Egypt, who are highly skilled in this area and whose ages range from 25 to 57, were invited to participate in the sensory analysis session in order to assess each cheese sample based on sensory acceptance. It was trained to do the sensory analyses at storage days 0, 10, 20, and 30. Under the panel leader's guidance, the chosen assessors created sensory language and described the products' sensory attributes. These skilled panelists assessed the samples for flavor, overall appearance, body, and texture. Panelists are trained to recognize intensity, quality, and temporal aspects of attributes. The organoleptic scoring system was used to evaluate cheese samples based on their outer appearance (20 points), taste and flavor (40 points), body and texture (40 points), and a combination of scores for several attributes (total score) according to the scorecard [12]. Each judge received three cheese cubes for each sample. Water

and non-salted crackers were given to them to cleanse their palates in between samples. Panelists are given clear and detailed instructions before the evaluation begins. They are informed about the purpose of the evaluation, the specific attributes to be evaluated, and the evaluation procedures. Panelists are instructed to refrain from consuming any strongly flavored substances, such as coffee or spicy foods, prior to the evaluation to prevent lingering effects on their sensory perception. Panelists should have no allergies or other conditions that may affect their sensory perception.

Statistical evaluation

The GLM technique was used for statistical analysis utilizing SAS [49] software. The means were compared using analysis of variance (ANOVA) and Duncan's multiple comparison approach. All experiments in this study were done in triplicate and the results were averaged. The significance of differences was set at $p < 0.05$.

Results and discussion

The phenolic compounds and water soluble vitamins content in spring onion extract

Phenolic compounds are known for their antioxidant properties and potential health benefits [15]. Furthermore, water-soluble vitamins are vital micronutrients that play critical functions in the body's physiological activities. Table 1 shows the phenolic compounds and water-soluble vitamin content in spring onion extract, determined using high-performance liquid chromatography (HPLC) in micrograms per gram ($\mu\text{g}/\text{g}$). Among the phenolic compounds, chlorogenic acid was found to have the highest concentration (1021.22 $\mu\text{g}/\text{g}$), followed by gallic acid (915.83 $\mu\text{g}/\text{g}$), syringic acid (452.73 $\mu\text{g}/\text{g}$), ellagic acid (215.98 $\mu\text{g}/\text{g}$), methyl gallate (187.102 $\mu\text{g}/\text{g}$), and naringenin (118.73 $\mu\text{g}/\text{g}$). These compounds are well-known for their antioxidant and anti-inflammatory characteristics, as well as their ability to lower the risk of chronic illnesses including cardiovascular disease, cancer, and neurological disorders [6, 50]. Other phenolic compounds present in smaller amounts, less than 100 micrograms per gram, include caffeic acid, cinnamic acid, kaempferol, coumaric acid, ferulic acid, daidzein, rutin, and quercetin. Although they are present in smaller amounts, they still contribute to the overall polyphenol content of spring onion extract, and their presence in spring onion extract suggests that it may have potential health benefits. Table 1 shows that among the water-soluble vitamins, vitamin C was found to have the highest concentration (771.15 $\mu\text{g}/\text{g}$). Vitamin C is a powerful antioxidant that

Table 1 HPLC analysis of phenolic compounds and water soluble vitamins in spring onion extract

| Items | Peak area | Conc. ($\mu\text{g/g}$) |
|-------------------------------|-----------|---------------------------|
| Phenolic compounds | | |
| Gallic acid | 305.54 | 915.83 |
| Chlorogenic acid | 179.52 | 1021.22 |
| Methyl gallate | 83.04 | 187.10 |
| Caffeic acid | 25.02 | 74.89 |
| Syringic acid | 148.46 | 452.73 |
| Rutin | 9.30 | 43.95 |
| Ellagic acid | 20.70 | 215.98 |
| Coumaric acid | 35.70 | 36.76 |
| Ferulic acid | 6.45 | 13.53 |
| Naringenin | 31.40 | 118.73 |
| Daidzein | 11.98 | 28.98 |
| Quercetin | 4.49 | 21.73 |
| Cinnamic acid | 69.04 | 52.71 |
| Kaempferol | 2.25 | 13.48 |
| Water soluble vitamins | | |
| Vit C | 476.8 | 771.15 |
| Vit. B1 | 19.87 | 36.31 |
| Vit. B2 | 10.72 | 7.40 |
| Vit. B6 | 71.92 | 254.85 |
| Vit. B12 | 20.27 | 128.65 |

aids in immunological function, collagen formation, and iron absorption [9, 26]. Spring onion extract was also found to be a good source of vitamin B6, with a concentration of 254.85 $\mu\text{g/g}$. Vitamin B6 is involved in numerous metabolic processes, including amino acid synthesis, neurotransmitter production, and haemoglobin formation [13, 37]. Other water-soluble vitamins present in smaller amounts include vitamin B12, vitamin B1, and vitamin B2. Overall, the results suggest that spring onion powder is a good source of various phenolic compounds and various water-soluble vitamins, particularly vitamin C and vitamin B6. Similar results were obtained by Aquino et al. [5, 10, 52], who established that vegetables such as spring onions are rich sources of water-soluble vitamins and phenolic compounds.

The chemical composition of spring onion extract fortified cheese treatments

Table 2 represents the chemical composition of fresh spring onion-fortified cheese treatments. The results show that the moisture content of all the cheese treatments decreased slightly ($p < 0.05$) from 73.30 to 72.45% with the addition of a spring onion extract of cheese. So on, the total solids (T.S.) content of the cheese treatments also did vary faintly, ranging from 26.70 to 27.55%. This difference between treatments in moisture and total solids content may be due to the addition of spring onion extracts, which are incorporated into UF-soft cheese at levels of (0.1, 0.3, and 0.5%).

Comparing the different treatments, the protein and fat contents of the cheese treatments were similar, ranging from 9.08 to 9.43% and 9.00–9.75%, respectively. The highest value of protein and fat content was noted in T3, which was supplemented with a higher amount of spring onion extract. Moreover, there is no significant impact ($p > 0.05$) on the carbohydrate content between cheese treatments. These results suggest that spring onion fortification did not have a significant impact on the fat and carbohydrate content of the cheese. However, the ash content of the cheese treatments did slightly differ, ranging from 2.62 to 2.71%. These small variations in the ash content suggest that spring onion fortification may have a slight impact on the mineral content of the cheese.

Changes on TS, pH and SN/TN values of cheese treatments

Figure (1 a, b, and c) represent the change in total solids (TS), pH, and SN/TN values in different spring onion fortified cheese treatments during various storage periods. Total solids are an important quality parameter of cheese, which includes the sum of protein, fat, carbohydrates, and ash in the cheese. Figure (1a) shows that the total solids increased in all the cheese treatments with the progression of the storage period. This increase in total solids is likely due to moisture loss during ageing, which causes an increase in the

Table 2 The chemical composition of spring onion extract fortified cheese treatments

| Treatments | Moisture% | T.S% | Protein% | Fat% | Ash% | Carbohydrate % |
|------------|----------------------------------|-------------------------------|---------------------------------|---------------------------------|-------------------------------|---------------------------------|
| C | 73.30 ^A ± 0.05 | 26.70 ^B ± 0.04 | 9.08 ^B ± 0.01 | 9.00 ^A ± 0.03 | 2.71 ^A ± 0.50 | 5.91 ^A ± 0.04 |
| T1 | 73.19 ^A ± 0.04 | 26.81 ^B ± 0.06 | 9.10 ^B ± 0.14 | 9.40 ^A ± 0.04 | 2.69 ^{AB} ± 0.13 | 5.92 ^A ± 0.08 |
| T2 | 72.67 ^B ± 0.10 | 27.33 ^A ± 0.03 | 9.36 ^A ± 0.17 | 9.50 ^A ± 0.05 | 2.66 ^{AB} ± 0.50 | 5.81 ^A ± 0.06 |
| T3 | 72.45 ^B ± 0.03 | 27.55 ^A ± 0.11 | 9.43 ^A ± 0.09 | 9.75 ^A ± 0.05 | 2.62 ^B ± 0.25 | 5.75 ^A ± 0.05 |

Data expressed as average of three replicates \pm SD. C: control cheese without extract; T1: cheese with 0.1% spring onion extract; T2: cheese with 0.3% spring onion extract; T3: cheese with 0.5% spring onion extract. Means in the same column showing the same capital letters are not significantly different ($p > 0.05$)

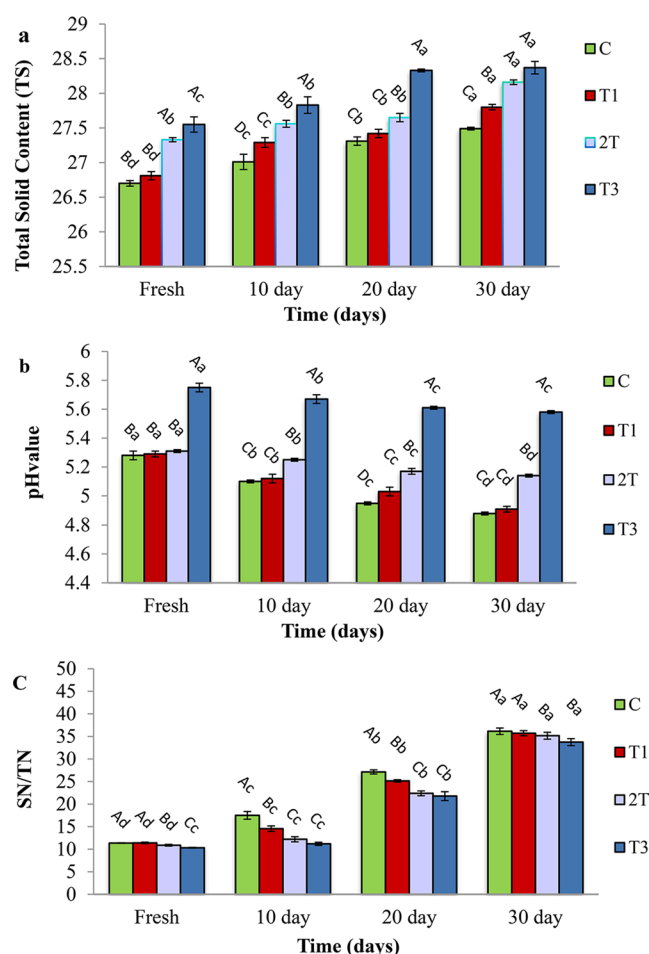


Fig. 1 a, b, c: Changes in total solids (TS), pH, and SN/TN values in different spring onion fortified cheese treatments during various storage periods. Data expressed as average of three replicates \pm SD. Means in the same capital superscript letters (effect of add extract) are not significantly different ($p > 0.05$). Means in the same small superscript letters (effect of storage) are not significantly different ($p > 0.05$)

concentration of the other ingredients in the cheese. Comparing the different treatments, treatment T3 consistently had the highest total solids during storage. Furthermore, the differences in total solids between the different storage periods were relatively small but significant. This suggests that the total solids of the cheese may have reached a plateau after 30 days of storage. The total solids content of the fresh cheese treatments also did vary faintly, ranging from 26.70 to 27.55%. Spring onion extracts typically have a negligible impact on the solids content of soft cheese. Spring onion extracts added to the cheese formulation, their contribution to the overall solids content is minimal. Therefore, the direct effect of spring onion extracts on the solids content of soft cheese is slight.

Figure (1b) represents the change in pH values in different spring onion fortified cheese treatments during various storage periods. pH is an important quality parameter

of cheese that affects the texture, flavor, and shelf life of the cheese. The pH values decreased significantly ($p < 0.05$) in all the cheese treatments with the progression of the storage period. This decrease in pH is likely due to the production of lactic acid by lactic acid bacteria during cheese aging. There were differences in the pH values between the treatments. For instance, treatment T3 consistently had the highest pH values during storage. This, due to the spring onion fortification, may have a significant impact on the increase in pH values in cheese. Alberto et al. [2] established that lactic acid bacterial growth was encouraged by phenolic compounds. Furthermore, Spring onion extracts can potentially affect the pH value of soft cheese. Spring onion extracts may contain organic acids that can influence the pH of the cheese [35]. These organic acids may decrease the pH, depending on their concentration and the buffering capacity of the cheese matrix. Figure (1c) represents the change in the ratio of soluble nitrogen to total nitrogen (SN/TN) in different spring onion fortified cheese treatments during various storage periods. The SN/TN ratio is an important indicator of cheese quality and can provide insight into the proteolysis process that occurs during cheese aging. The figure shows that the SN/TN ratio increased in all the cheese treatments with the progression of the storage period. This increase in the SN/TN ratio is expected as cheese undergoes proteolysis during storage, which results in the breakdown of proteins into smaller peptides and amino acids [25]. The increase in the SN/TN ratio indicates that there was an increase in the proportion of soluble nitrogen (peptides and amino acids) relative to total nitrogen (proteins) during storage. Comparing the different treatments, the control treatment consistently had the highest SN/TN ratio values during storage, and there were few differences in the SN/TN ratios between the treatments. This recommends that the level of spring onion fortification may have a small but significant impact on the proteolysis process that occurs during cheese storage. Spring onion extract contains enzymes such as alliinase, which can exhibit proteolytic activity [11, 32]. These enzymes can interact with the proteins in cheese and facilitate their breakdown, leading to increased proteolysis.

The DPPH and ABTS values of spring onion extract fortified cheese treatments during storage periods

Figure 2(a and b) shows the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS values of spring onion fortified cheese treatments during different storage periods. DPPH and ABTS are widely used antioxidant assay methods that measure the ability of a substance to scavenge free radicals. Figure (2a) shows that the DPPH values are expressed in terms of percentage inhibition, which means that a higher value indicates a better antioxidant potential of the sample.

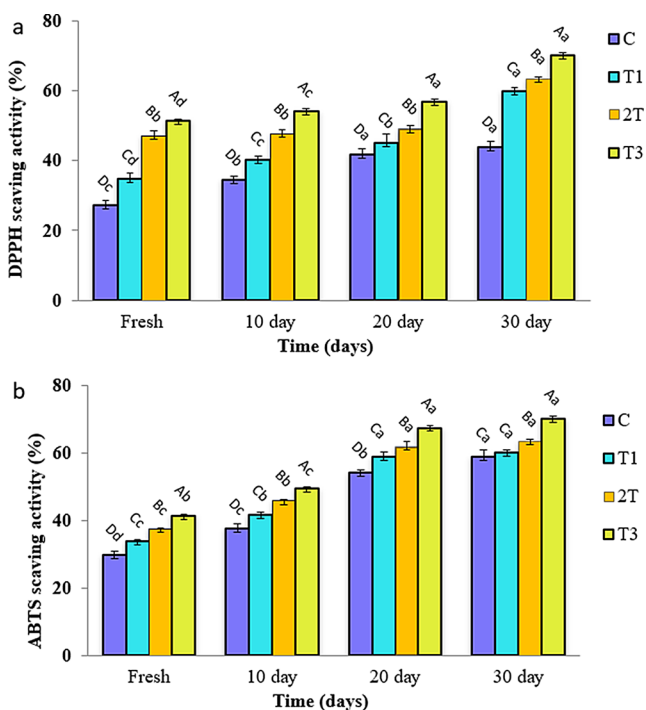


Fig. 2 a, b: DPPH and ABTS values of spring onion extract fortified cheese treatments during storage periods. Data are represented as mean of three replicates \pm SD. Means in the same capital superscript letters (effect of add extract) are not significantly different ($p > 0.05$). Means in the same small superscript letters (effect of storage) are not significantly different ($p > 0.05$)

During the storage, all the treatments show an increase ($p < 0.05$) in DPPH values as the storage period increases from fresh to 30 days. This is expected as the oxidation process progresses with time, leading to an increase in free radical production [28]. Comparing the treatments, T3 has the highest DPPH values at all storage periods, indicating the highest antioxidant potential. T2 also shows a noticeable increase in DPPH values compared to T1, indicating that the higher fortified amount of spring onion extract has a positive effect on the antioxidant potential of the cheese.

In the same way, the same Fig. (2b) shows that the ABTS values of all the cheese treatments increased ($p < 0.05$) with the progression of the storage period, indicating an increase in the antioxidant activity. This is expected, as the natural antioxidants in spring onion extract would have diffused into the cheese matrix, thereby increasing its antioxidant activity. Among the different treatments, it is apparent that the cheese samples fortified with higher levels of spring onion extract (T3) had the highest ABTS values compared to the samples with lower levels of spring onion (T2, T1, and C). This suggests that the level of spring onion fortification has a significant impact on the antioxidant activity of the cheese. These results are in line with earlier research that showed a comparable pattern in the addition of natural antioxidants to other food products (Karadeniz, 2005;

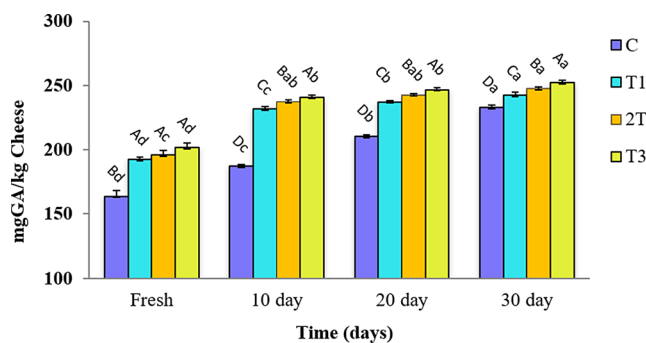


Fig. 3 Total phenol content of spring onion extract fortified cheese treatments during storage periods. Data are represented as mean of three replicates \pm SD. Means in the same capital superscript letters (effect of add extract) are not significantly different ($p > 0.05$). Means in the same small superscript letters (effect of storage) are not significantly different ($p > 0.05$)

Pereira & Tavano [42], . Overall, the results suggest that spring onion fortification can be an effective strategy to increase the antioxidant activity of cheese, with concern for the optimal level of spring onion fortification that provides the maximum antioxidant benefit without compromising the quality of the cheese.

Total phenol content of cheese

TPC is a commonly used assay that measures the total amount of phenolic compounds in a sample. Phenolic compounds are natural antioxidants that have been shown to have health-promoting properties (El-Sayed & Youssef [19]. Figure 3 represents the total phenol content (TPC) values of different spring onion extract-fortified cheese treatments during various storage periods. The TPC values of all the cheese treatments increased significantly ($p < 0.05$) with the progression of the storage period, indicating an increase in the phenolic content.

This is expected, as the natural phenolic compounds in spring onion extract would have diffused into the cheese matrix, thereby increasing its phenolic content [47]. The cheese samples fortified with 3% of spring onion extract (T3) had higher TPC values compared to the control samples and other samples with lower levels of spring onion (T1 and T2). This suggests that the level of spring onion extract fortification has a significant ($p < 0.05$) impact on the phenolic content of the cheese.

The color values in different spring onion extract fortified cheese treatments

The color values are an important indicator of cheese quality and can provide insight into the changes in color that occur during cheese ageing. Table 3 represents the change in the L^* , a^* , and b^* values in different spring onion

Table 3 Change in the color measurements in different spring onion extract fortified cheese treatments during storage periods

| Color value | Storage (days) | Treatments | | | |
|-------------|----------------|---------------------------|----------------------------|---------------------------|----------------------------|
| | | C | T1 | T2 | T3 |
| l* | Zero | 88.60 ^{Aa} ±0.10 | 88.30 ^{Ba} ±0.10 | 86.60 ^{Ca} ±0.10 | 86.10 ^{Da} ±0.20 |
| | 10 | 85.87 ^{Ab} ±0.25 | 84.87 ^{ABb} ±0.15 | 83.70 ^{Bb} ±1.21 | 82.23 ^{Cb} ±0.15 |
| | 20 | 79.97 ^{Bc} ±1.61 | 80.00 ^{Bc} ±0.10 | 81.63 ^{Ac} ±0.15 | 81.40 ^{ABc} ±0.10 |
| | 30 | 78.50 ^{Bc} ±1.65 | 79.60 ^{ABd} ±0.10 | 80.17 ^{Ad} ±0.15 | 80.50 ^{Ad} ±0.10 |
| a* | Zero | 0.27 ^{Db} ±0.06 | 1.57 ^{Ca} ±0.32 | 2.77 ^{Ba} ±0.06 | 3.67 ^{Aa} ±0.38 |
| | 10 | 0.37 ^{Dab} ±0.06 | 1.43 ^{Cab} ±0.06 | 2.50 ^{Bb} ±0.10 | 3.30 ^{Aab} ±0.30 |
| | 20 | 0.50 ^{Da} ±0.10 | 1.17 ^{Cb} ±0.06 | 1.70 ^{Bc} ±0.10 | 3.03 ^{Ab} ±0.25 |
| | 30 | 0.50 ^{Da} ±0.10 | 0.93 ^{Cc} ±0.15 | 1.67 ^{Bc} ±0.06 | 2.03 ^{Ac} ±0.15 |
| b* | Zero | 14.97 ^{Ca} ±0.15 | 22.87 ^{Ba} ±0.25 | 31.60 ^{Aa} ±1.35 | 33.20 ^{Aa} ±1.45 |
| | 10 | 15.07 ^{Da} ±0.21 | 21.37 ^{Cb} ±0.25 | 24.93 ^{Bb} ±0.15 | 29.03 ^{Ab} ±0.83 |
| | 20 | 15.00 ^{Da} ±0.10 | 19.90 ^{Cc} ±0.20 | 24.70 ^{Bb} ±0.10 | 27.97 ^{Ab} ±0.15 |
| | 30 | 15.03 ^{Da} ±0.06 | 18.20 ^{Cd} ±1.35 | 22.03 ^{Bc} ±0.06 | 24.53 ^{Ac} ±0.15 |

The data are shown as the standard deviation of three replicates. The means of the identical little characters in various rows (impact of storage) are not significantly different ($p > 0.05$). The means for columns with the same capital letters (impact of adding extract) are not statistically distinct ($p > 0.05$). The l^* value ranges from black (0) to brightness (100). The a^* value indicates the red color (positive) to the green color (negative), the b^* value indicates the yellow color (positive), and the negative value indicates the blue color

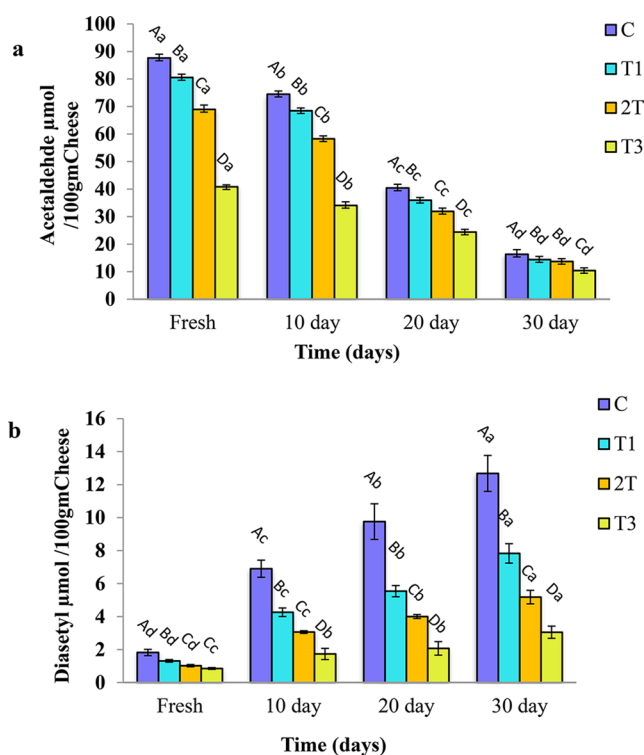


Fig. 4 a, b Acetaldehyde and diacetyl values of spring onion extract fortified cheese treatments during storage periods. Data are represented as mean of three replicates±SD. Means in the same capital superscript letters (effect of add extract) are not significantly different ($p > 0.05$). Means in the same small superscript letters (effect of storage) are not significantly different ($p > 0.05$)

extract-fortified cheese treatments during various storage periods. The l^* , a^* , and b^* values decreased significantly ($p < 0.05$) in all the cheese treatments with the progression of the storage period.

The l^* value decreases, indicating darker cheese; the a^* value decreases, indicating less red and extra green cheese; and the b^* value decreases, indicating less yellow and extra blue cheese. This is predictable due to proteolysis or lipid oxidation during the storage period, which can cause color changes. This indicates that cheese quality and color changes are influenced by the storage period. Comparing the different treatments, the variances in l^* , a^* , and b^* values between different cheese treatments were representative of the fact that color changes may have increased with the addition of spring onion extract, particularly for the a^* and b^* values of T3, which were consistently higher than the other treatments during storage. According to the visual look, the spring onion extract has a deeply green colour, which may be responsible for the green color of fortified cheese products. This suggests that the extra fortification of spring onion extract may have a significant impact on the color changes that tend to show a larger saturation of the green and yellow colors.

Aroma components of fortified cheese

Figure 4(a, b) represents the acetaldehyde and diacetyl values of different spring onion extract fortified cheese treatments during various storage periods. Acetaldehyde and diacetyl are organic compounds that are naturally present in cheese and are responsible for its flavor and aroma. Figure 4a shows that the acetaldehyde of all the cheese treatments decreased significantly ($p < 0.05$) with the progression of the storage period, contrary to the fact that diacetyl values increased ($p < 0.05$), as shown in Fig. 4b. The decrease in acetaldehyde values over time is expected as these compounds are volatile and can evaporate from the cheese matrix during

storage. Furthermore, research by Lees & Jago [34, 51] has shown that if high acidity is present during the lactic acid bacteria's growth during storage, cheese maturing may produce acetaldehyde and other flavor compounds at a slower rate. Diacetyl production increases during storage due to LAB growth and natural pathways involving α -acetolactic acid [39]. Moreover, the levels of diacetyl depend on fermentation performance and conditions like pH, oxygen, and temperature [22, 41]. Comparing the different treatments, it is observed that the cheese samples fortified with higher levels of spring onion extract (T2 and T3) had lower acetaldehyde and diacetyl values compared to the samples with other treatments. This suggests that the level of spring onion extract fortification has a significant impact on the flavor and aroma profile of the cheese.

Sensory properties of cheese treatments

Figure (5 a, b, c, and d) discuss the changes in scores for the sensory attributes of different spring onion extract-fortified cheese treatments during various storage periods. These treatments were evaluated based on their sensory attributes, specifically their surface appearance, body and texture, taste and flavour, and the total scores were recorded at different storage periods: fresh, 10 days, 20 days, and 30 days. Figure (5a) indicates that all the cheese treatments experienced a decline in surface appearance scores over the storage period. However, the rate of decline varied slightly among the treatments, with control and T1 showing a relatively faster decline compared to T2 and T3. This information suggests that the increased addition of spring onion extract to the cheese treatments may not have influenced their surface appearance during storage.

Figure (5b) shows the "body and texture" scores, and it is noticeable that all four treatments (C, T1, T2, and T3) started with an initial score of 35 for the fresh cheese. As the storage period progressed, it was observed that the scores generally increased for fortified treatments, indicating further enhancement in the body and texture of the cheese over time, such as smoother and easier spread. On the other hand, the control treatment displayed a relatively stable score across all storage periods. Treatment T3 demonstrated a consistent increase in scores as well. Moreover, Fig. 5b indicates that T3 also experienced an improvement in body and texture over time, albeit at a slightly higher rate compared to T1 and T2.

Figure (5c) presents the changes in scores for the sensory attribute of "taste and flavor" in different spring onion-fortified cheese treatments during various storage periods. The taste and flavor of all the cheese treatments improved during the storage period. T2 had a notable improvement in taste and flavor, with higher scores throughout the storage

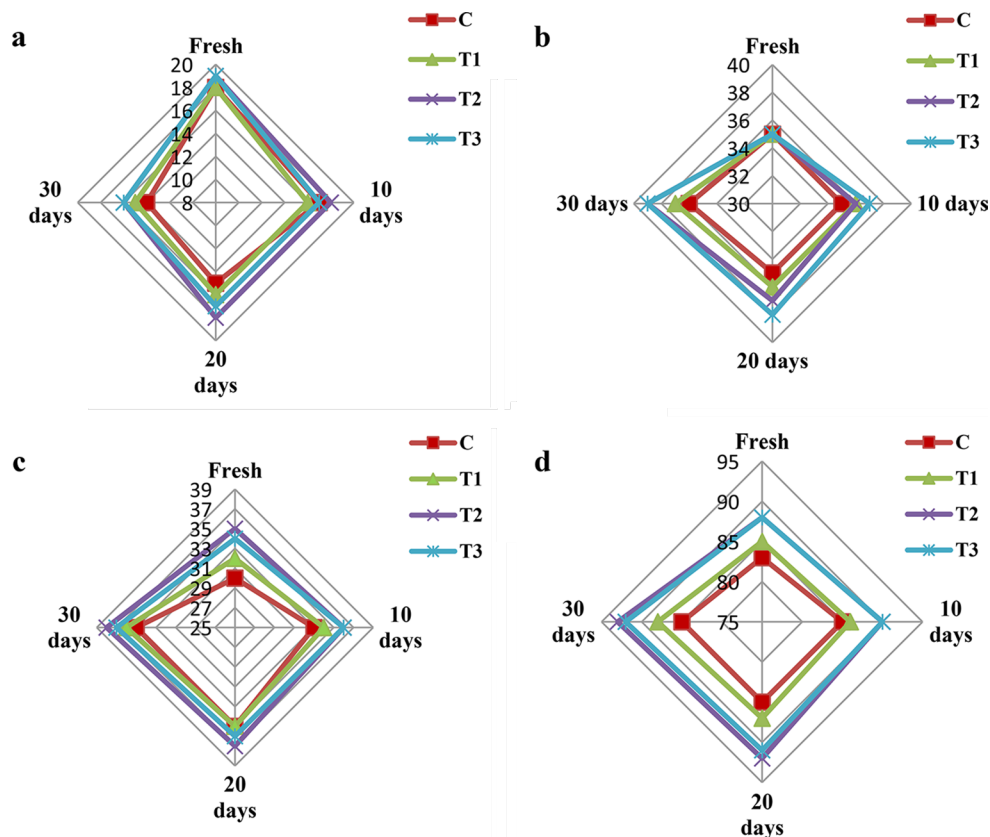
period. Treatments T1, T2, and T3 consistently exhibited higher total scores compared to the control treatment (C). T2 started with an initial score of 35 for the fresh cheese, signifying a higher taste and flavor quality compared to other cheese treatments.

Figure (5d) illustrates the "total score" attribute of different spring onion-fortified cheese treatments during various storage periods. Treatments T1, T2, and T3 consistently exhibited higher total scores compared to the control treatment (C), suggesting potential enhancements in sensory attributes for the fortified treatments. T2 and T3, in particular, showed notable improvements in the total score, indicating an overall increase in sensory appeal. These are mainly related to the suitable amount of spring onion extract that matches cheese with color, body, texture, and flavor.

Conclusion

Spring onions are rich in nutrients like vitamins, minerals, dietary fiber, antioxidants, and bioactive substances. This study focuses on applying spring onion extract fortification in cheese as a functional product with naturally antioxidant compounds. The highest concentrations of chlorogenic acid and vitamin C in the extract were found. This suggests that fortifying cheese with spring onions extract could increase antioxidant activity and phenolic content over various storage periods, potentially replacing synthetic antioxidants in the food industry. The cheese samples fortified with more spring onion extract showed reduced acetaldehyde and diacetyl levels. Fortification with spring onion extract also significantly influenced the cheese's pH and SN/TN ratio, indicating cheese quality. During storage, the taste and flavor of all cheese treatments were enhanced, with T2 having the best taste and flavor. The total sensory score for cheese is listed in descending order of acceptability: T2 > T3 > T1 > C.

Fig. 5 a, b, c, d: Changes in scores for the sensory attributes of different spring onion fortified cheese treatments during various storage periods. Data expressed as average of three replicates \pm SD. C: control cheese without spring onion extract; T1: cheese with 0.1% spring onion extract; T2: cheese with 0.3% spring onion extract; T3: cheese with 0.5% spring onion extract. a: Surface appearance (20), b: Body & Texture (40), c: Taste & Flavor (40), d: Total score(100)



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Data availability The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding author/s.

Declarations

Ethical approval This work was approved by the Medical Research Ethics Committee (MREC), National Research Centre, Cairo, Egypt with approval number Ex-08411123, and followed the recommendations of the National Institutes.

Conflict of interest No potential conflict of interest was reported by the authors.

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