Preparation of a Micro laminate of alginate and Cinnamon in LBL Technique and its use in Coating of Cheddar

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Abstract. This study was carried out to evaluate the preparation of five-layer microlaminates that were introduced by Layer By Layer (LBL) technique by the use of two solutions, namely, the sodium alginate, and the other antimicrobial agent is the Cinnamon extract. The scanning electron microscope was used to detect the thickness of prepared microlaminates. The thickness of the total alginate and Cinnamon microlaminate was 22.47 µm. The Zeta Potential voltage of the alginate solution reached -28.49 mV at pH = 7 and the Cinnamon extract was 28.69 mV. The WVP water permeability values for the microlayered PET film without any addition to the charged PET (treatment 1) and for the microlayered PET-charged for sodium alginate and Cinnamon extract (treatment 2) 29.091 g.m2/24h, OTR was obtained for the nanolayered with no addition of the charged PET (treatment 1), 14.78 ml / m2.day), and for the PET-charged, covered with sodium alginate and Cinnamon extract (treatment 2) 17. 95 ml /m2.day). Three treatments were made of cheddar cheese, the first treatment was covered with the paraffin wax as control M1, the second was covered with gelatin (M2) and the third was coated with a microlayered film consisting of the sodium alginate and the Cinnamon extract (M3). The results showed a significant decrease in the moisture content and the acidity of the treatment M3 and increase in the values of ADV during period storage, and using the Cinnamon extract in the microlayered was making it superior in the sensory characteristics of the comparison treatments.

1. Introduction
The production of high quality food with long-term quality and quality characteristics is itself one of the main challenges in food processing. To achieve this goal, the edible crust is used on the surface of the food. It is composed of natural polymers with functional properties such as gas tolerance and antibacterial action which have improved the quality and safety of food, and recently one of the challenges in the area of the edible casing is the use of layer-over-layer technology to overcome some problems related to the permeability of high water vapor and poor mechanical properties of the edible crust compared to those Manufactured from synthetic materials. Some of the benefits of using this microcosmetic are high stability on the material surface and low concentration of materials required [1;2], a technique that is used in the manufacture of nanoparticles and microcircuits, has been applied in various fields such as biomedicine and food processing. To the fact that the microcosm consists of two or more layers of materials with nanoscale or micro nuclear dimensions and some chemical or physical bonds that improve the water retention properties of the starch that enters into the edible
envelope industry to protect food against microbial damage taking into account the properties of the envelope. Functionality of water content Layer By Layer (LBL) technology as a suitable way to obtain a microcosm of natural polymers suitable for consumption and used in food coating, which must be electrostatic charged with important functional properties such as antibiotics, antioxidants and functional gas loading properties [3], and has never been used on processed cheese, which is a complex food product consisting mainly of water, casein and fat, as well as a large consumption product.

2. Materials and Methods:

2.1 PET:
Poly Ethylene Terephthalate (PET) was obtained from Sigma Aldrich, a German company with a thickness of 0.005 nm, as a supporting membrane for the solution of its coating solutions when studying its properties.

2.2 Preparation of solutions

2.3 Preparation of the Cinnamon extract

The extraction process was carried out according to Preparation of the coating solutions used in the Cheddar cheese coating: I attended the coating solutions used in the cheese coating according to the method described by [3] and modified by us included: Sodium Alginate Solution: Prepare 0.2% (weight / volume) to dissolve the sodium alginate (equipped with Hi Media Lab) in distilled water and using a magnetic mixer at 200 rpm for two hours at 70 °C, Then at 20 °C for 22 hours, pH of the alginate solution was adjusted to 7 using a NaOH solution (1 molar) and performed with a ultrasonic ultrasonic homogenizer with 80 pulses for 4 minutes and stored in the refrigerator until use. Solution of plant extract of Cinnamon: Attended the same method as preparation of the sodium alginate solution, but adjusted the pH to 3.8 using lactic acid 1 molar. Gelatin Solution: Prepare the gelatin solution with a concentration of 10% according to the method mentioned by [5].

2.4 Measuring the efficacy of flavonoid extract for Cinnamon antacid by estimating the value of peroxide value in sunflower oil

The process of estimation according to the steps mentioned by [6]. by adding different concentrations of the ethylene extract of the Cinnamon 50, 100 (ppm) to 20 ml of the sun flower oil supplied from the local markets, In the air oven at 85 °C for 1 (day)

2.5 Preparation of the micro laminate

The laminate was potting repaired for the purpose of describing it in two phases. The first phase included the loading of polymer polyethylene (PET) polyethylene as a supporting membrane for the solution of the coating solutions when studying its properties. The method was carried out as described by [7] (1: 1) for 3 hours, then rinse with distilled water and blow at 30 °C for 24 hours, and then cover with 0.06 g of 1.6 hexandiamine / Propanol at 37 °C for 4 hours and after the specified wash period. With distilled water to dispose of the above material and dry at 37 °C for 24 hours, the charged coating was treated with 0.1 mL HCl for 3 hours at a temperature of (2 x 1 cm) and immersed in the alginate solution for 15 minutes, then washed with ions-free water, and then washed with a large amount of distilled water and dried at 30 °C for 24 hours. PH 7 and similar to the pH of the alginate solution and left until dry and then dipped with the solution of the plant extract of the Cinnamon and for 15 minutes after it was washed with acidic acid water of pH3.8 and similar to the pH of the flavonoid extract. The process was repeated five times to form five alternating layers (alginate - alginate extract - alginate extract) at 20 °C and 50% relative humidity.

2.6 Characterization of the microlaminate

Fourier Transform spectroscopy (FTIR): The FTIR measurement was carried out at wavelengths of 4000-650 cm - 1 to examine the active aggregates of the charged and unpacked PET surfaces of the Cinnamon plant extract and placed on the lens of the sensitive apparatus at room temperature.

Zeta Potential: Zeta Potential for alginate solutions and the Cinnamon extract was calculated at the Nanotechnology Research Center at the University of Technology using the Nano book Zeta plus zeta potential analyzer [8].
UV-visible spectrometer: The absorption of UV spectrometry was measured using the optical spectrometer and was used for multi-prepared and absorbent coatings at a wavelength of 260 nm [9]. Water vapor permeability measurement (WVP): WVTR was measured according to the US classification [10] at the Industrial Research and Development Center / National Center for Coating and Coating.

Measuring oxygen permeability rate (OTR): The oxygen permeability rate of the coating was tested using the OTR device at the Industrial Research and Development Center / National Coating and Coating.

Scanning electron microscopy (SEM): The examined envelope was examined on the 5-layer charged PET surface as well as the non-charged PET surface of the scanner electron microscope at the Nanotechnology Research Center at the University of Technology.

2.7 Manufacturing Cheddar cheese

The method mentioned by [11] was adopted in the manufacture of all Cheddar cheese

2.8 Coating of Cheeses

The method of dipping was followed to coating the cheeses as follows: To prepare the treatment 3M, cut the cheese (the weight of the piece 75 g) with distilled water and leave to dry for 20 minutes at 25 °C, then submerge in the alginate solution. The sodium is 15 minutes cold and left to dry for 20 minutes. Which is the same as the pH of the sodium alginate solution. It was then submerged with the Cinnamon solution of pH = 3.8. The process was repeated with the sodium alginate solution and the Cinnamon solution. In the previous conditions, Compared to T1, T2, T1 treatment was used in wrapping paraffin-equipped wax to The dairy company, Abu Ghraib, Baghdad, where the cheese was immersed in dissolved molten wax solution at 118 °C for 5 seconds and then removed to dry. Treatment of T2 was wrapped in gelatin envelope. After drying the cheese pieces, the gelatin solution was mixed with stirring between time and time. After the coating process was completed, the samples were placed in sealed, sterile plastic containers and stored in the refrigerator at 10 ± 2 °C for 2 months for the.

2.9 Cheese tests

Determination the percentage of moisture lost

Moisture ratio lost from uncoated cheese - Moisture ratio lost from coated cheese

\[
\frac{\text{Percentage of moisture lost}}{\text{Moisture ratio lost from uncoated cheese}} \times 100
\]

Cheddar cheese for chemical and sensory tests: The method, mentioned by [12] and modified by [13], was used to estimate the percentage of moisture. The percentage of ash was estimated by burning the samples at the Muffle furnace at 550 °C for 6 hours or until the weight was stable. [14], the pH according to the method described in [15] was estimated as the total nitrogen count according to the method described by [12], the soluble nitrogen and non-protein nitrogen (NPN) according to the method described in [16], the Acid Degree Value (ADV) was estimated in the Bureau of Dairy Industry (BDI) cited by [17].

2.10 Sensory evaluation of cheese

The sensory evaluation of the Cheddar cheese samples from professional evaluators based on the sensory assessment forms derived from the proposals for edible endotracheal applications proposed by [18] compared to the control coated with paraffin wax and gelatin-coated cheese, Notes for paint condition and other qualities.

2.11 Statistical Analysis

The Statistical Analysis System (SAS) [19] was used.
3. Results and discussion:

3.1 Antioxidant activity of the extract

Study the effect of adding the extract of the researchers after adding to the alginate used in the preparation of multi-layer multi-layer polymeric coatings in preventing or delaying the oxidation process. The results showed that the value of the peroxide number was 5.8 mol/kg when using the 50- With a concentration of more than 100 parts per million (5.5 meq/kg), indicating that there is no effect of increasing the concentration of the extract on the effect of the oxidation processes. This is confirmed by the results of the statistical analysis, as the value of LSD, which indicates the value of the least significant difference between the concentrations on the level of probability of P > 0.05 used was 6.07. Based on the above, a concentration of 0.2% of the plant extract can be used in the preparation of micron micro laminate by combining them with the same concentration and use in the cheese coating.

3.2 Study of the physical properties of the casing and the polycarbonate solutions of the casing

3.2.1 Analysis of the infrared spectrum FTIR: Use the FTIR infrared measurement to confirm the presence of active aggregates on the PET surface. Fig. 1 shows the FTIR spectra of the charged PET membrane only and without addition, by having its peaks at 1719 and 1249 cm<sup>-1</sup> And 1103, which are related to the groups of carboxylic ring, and the summit, whose location 727 cm<sup>-1</sup>, returns to the aromatic (CH) aroma [7 and 20]. Figure (2) shows the results of the FTIR analysis of the researcher's extract, noting the emergence of the amplitude at 1096 cm<sup>-1</sup>, which results from the characteristic decomposition of COC, with two peaks located at 1715 and 1244 cm<sup>-1</sup>. [21] that an IR packet at 1666.7 cm<sup>-1</sup> belongs to the group OH and C = O and the package at 1000 cm<sup>-1</sup> back to C-OH The package at 1600 cm<sup>-1</sup> - returns to the group C = O and the package at 1580 cm -1 back to the group amide.

![Figure 1. Analysis of FTIR infrared spectra of the charged PET](image-url)
Figure 2. Analysis of the FTIR spectrum of the PET coated charged and encapsulated with alginate and Cinnamon

3.2.2 Zeta Potential Determination:
To study the electrostatic properties of alginate and plant extract solutions, the oil was calculated in Table 1, which was based on its comparison with the supporting PET surface. The oil voltage of the alginate solution was 28.49 mV and pH = 7 and 28.69 mV, the value of the negative oil voltage through the free carboxylic aggregates in the composition is pH 7. The water-loving surfaces depend on the nature of the outer layer and not on the base material. Usually sedimentation of the layers causes some effect on certain physical properties as well as changes in pH to have a significant impact on these changes can in turn affect the structure of the membrane itself. For example, the alginates prepared with high pH values have a low charge compared with those that have low pH values due to soft alginate surfaces [22]. The results were consonant with [3]. The values of the Zeta-62.13 ± 4.10 MV voltage for the sodium alginate solution at pH = 7 and -58.28 ± 4.18 mV for Chitosan solution at pH = 3.8 showed that it could interfere with electrostatic forces and the lysozyme was 29.27 ± 3.18 mV at pH = 3.8 with aggregates as well as with what [4] found. The values of the alveolar voltage of alginates were 62.13-mV at pH = 7 and for lysozyme at 25.67 ± 2.27 mV at pH = 3.8.

Table 1. Zeta Potential (mv) of the Cinnamon extract for the students *

| Zeta Potential (mv) | Type of solution or extract |
|--------------------|----------------------------|
| -28.49             | Alginate solution          |
| 28.69              | Cinnamon extract           |

*Measurements represent a rate of three readings.

3.2.3 UV-Vis Absorption:
In order to continue the assembly of the plant-made, alginate-formed coating layers on the PET surface, the photodynamic analysis was performed at a wavelength of 260 nm after each deposition. As the absorption value increases by depositing layer after layer, this confirms the successful deposition of the layers (Figure 3) shows the photovoltaic absorption at the wavelength of 260 nanometers of the multilayer layer of the alginate with the study extract, showing the increase in absorbance with the deposition of the five layers of each envelope, confirming the successful deposition of this processed micronized casing [3], which also found a significant increase in absorption values by depositing five layers of alginate and chitosan on the
surface of the charged PET. The images were confirmed by a SEM device. On the surface of the charged PET, [23] also reported that the increase in the number of layers of the casing caused a marked increase in the values of light absorption.

![Figure 3](image)

**Figure 3.** Spectral absorption of visible ultraviolet radiation to the Cinnamon extract.

### 3.2.4 Water permeability WVTR

The most important characteristic of the barrier in terms of its properties is its ability to hold water or its vapor and its ability to prevent the entry of gases such as carbon dioxide and oxygen into and out of food because of its impact on the quality and safety of food, [24]. The results of Fig. 4 where the WVP values indicate that the water vapor permeability of the charged PET only (treatment 1) is 29.091 g.m²/24h) and for the PET charged and alginate-encapsulated and the study extract 2 were similar with 29.091 g.m² 24h). Also, these results are good for the multilayer coating, which can depend on the self-interplay between the alginites and the antimicrobial agent antimicrobial agent layers. The results of the treatment (2) of the transplanted PET, It is for the charged PET only and this indicates that this casing containing the extract of the scholars has a value of pilgrimage G is very good due to its chemical composition. It is observed from the FTIR results of the original PET envelope that is only charged that it contains a single hydroxyl group at 2965 cm⁻¹ and then the case of treatment 2 which is also shown to contain a single hydroxyl group at 2965 cm⁻¹, They are close in terms of composition with a comparison treatment, which indicates the components of the casing that do not allow the permeability of water vapor molecules.

![Figure 4](image)

**Figure 4.** Water vapor permeability of the microlaminate of Cinnamon (g / m².24h)

Treatment 1 = PET charged

Treatment 2 = PET loaded and coated with alginites and the Cinnamon extract
3.2.5 Oxygen transfer rate (OTR):

OTR was obtained for the micronized membrane as shown in Figure (5) since the charged PET (treatment 1) was 14.78 ml / m².day) and the PET charged and coated with the alginate and the study extract (treatment 2) 17.95 ml / m².day). The results of the two treatments were of similar results, because of the nature of the chemical composition of the students, which reserves the amount of oxygen that is carried into the membrane as the oxygen is the first cause of food damage, which helps to save and increase the life of food and these results were higher than the values found [25] is 6.68 x 10^{-7} and 3.31 x 10^{-5} gm 2 tha-1 for corn and urinezinz membranes Ethylene respectively.

![Figure 5. The oxygen permeability of the microlaminate of Cinnamon (ml/m².day)](image)

Treat. 1= PET charged
Treat. 2 = PET loaded and coated with alginites and the Cinnamon extract

3.2.6 Scanning Electron Microscopic (SEM):

Figure 6 shows the images of the microscopic electronic survey (SEM) of different spectra of the multilayer micronosphere deposited on the surface of the PET Layer By Layer (LBL). The first layer consisted of five layers, Alternately, the alginate layer, which represents the upper surface layer, was soft and crystalline as shown in shapes (a and b). The same figure shows the appearance of multiple layers and different sizes of this envelope. In the first case the shape showed that the thickness of the total layers of the alginate The students were 22.47 m The result is consistent with what [27] found when using cellulose paper with an over-the-top layer in cooked beef and preserving the surrounding temperature, With [28] having found a network of cellulosic fibers with layers of carboxylic methyl cellulose /chitosan in a layer-layer pattern.
**Figure 6.** Scanning electron microscopy (SEM) images of PET coated with alginate and Cinnamon

a- picture of the layers of the microlaminte composed of alginate and Cinnamon

b- Of the surface using a scanning electron microscope

### 3.3 Chemical composition of cheese:

During the storage, the samples of the chemically coated macaroni cheese showed a decrease in weight loss (P <0.05) from 0 to 6 months for the cheese. The results shown in Figure 7 showed a significant decrease in the moisture content lost from the samples (Treated T3) and gelatin (treatment T2). This is due to the fact that the edible envelope, which uses multiple sugars and / or proteins, reduces the loss of mass of cheese. [1] (27) who showed Net (P <0.05) compared to non-coated cheese (comparison treatment) from 1 day to 20 days with 1.52 for the envelope cheese at the end of the storage period.

Loss of moisture

**Figure 7.** moisture loss during storage in the macaroni-encapsulated cheese digestion process for students during the 6-month maturation period and 10 ° C ± 2 °
T1: Cheddar cheese coated with wax

T2: Gelatinized cheese wrapped in gelatin envelope

T3: Cloves of coagulants made up of alginate and researchers

Moisture content: The results shown in Table (2) show the moisture content of cheese. The values at the beginning of ripening were within the limits of the moisture content set by the Iraqi Standard for Dry Cheeses [29]. They are 30-40% [30] showed a decrease in the moisture content values of all models with the age of ripening. The reason for this decrease is due to evaporation and loss of moisture from the cheese. Coated with the micron envelope was less than the amount of the drop in the moisture ratio of the paraffin-coated and gelatin-coated comparison cheese, this variation in wet moisture content is due to the fact that the casing contributes to moisture retention and prevents evaporation. This is an indicator of its efficiency in moisture retention during the storage period [8; 31].

Table 2. Percentages of moisture in the cheddar cheese coated with micro laminate of Cinnamon extract during the 6-month storage period at 10 °C ± 2 °.

| Treatments | % moisture in the cheese coefficients during storage ages (month) | LSD |
|------------|---------------------------------------------------------------|-----|
|            | 0 | 3 | 6 |                           |
| T1         | 38.66 | 36.94 | 31.00 | * 4.58                 |
| T2         | 38.70 | 34.12 | 29.00 | * 5.03                 |
| T3         | 38.67 | 36.54 | 34.09 | * 4.13                 |
| LSD        | 2.76NS | 2.08NS | * 3.91 | |

Titration acidity and pH: Acidity is in balance with other compounds to give the taste and flavor of cheese and thus affect the degree of consumer acceptance of foals [18]. The results of the statistical analysis showed a significant difference (p < 0.05) in pH values and age (Table 3). At the end of the storage period, pH values were observed in all stored refrigerants, ranging from 5.10 to 5.20 at the age of 8 weeks. These readings correspond to With the decline in the proportion of These proteins, which can be attributed to the degradation of these proteins by proteolytic enzymes derived from bacteria, renin and other microorganisms, produce base substances such as peptides, which play a role in reducing the acidity of cheeses [30]. [27] Found that in the case of Brazilian cheeses, a multilayered, multilayered, multicellular nucleic acid, the pH values increased in all treatments when stored for 21 days. The values rose from 5.22 to 7.12 in uncooked cheese. For packaged cheese rose from 5.29 to 6.47 this increase in pH values in coated coefficients was attributed to the analytical efficacy of the bacteria producing the proteolytic enzymes.

Acid Degree Value (ADV): The results indicate that the acidity of the fat of the coated cheeses consisting of alginate and T3 was 0.64 mg / kg base / 100 g fat at the beginning of the ripening period (Table 3). As the maturation process progressed, the results showed that the highest evolution in the ADV values was in T1 and T2 levels at 4.80 and 5.00 mAq / 100g while the development was lower in T3, which was 2.92 mAq / 100 g fat Refers to the role of this envelope in the retention of moisture within the cheese block, which provides a more favorable environment for initiatory bacteria activity, (Aw) to produce enzymes, especially lipid-converting enzymes.
Table 3. Percentage of acidity, pH values, and ADV in the cheddar cheese coated with micro laminate of Cinnamon extract during the 6-month storage period at 10 °C ± 2 °.

| Treatments | ADV (Mm/100 gm fat) | pH | Acidity (%) |
|------------|---------------------|----|-------------|
|            | 0   | 3 | 6 | 0 | 3 | 6 | 0 | 3 | 6 |
| T1         | 0.67 | 1.32 | 4.80 | 1.563* | 5.22 | 5.15 | 0.77 | 0.83 | 0.88 | 1.563 * |
| T2         | 0.65 | 1.35 | 5.00 | 1.669 * | 5.35 | 5.20 | 0.75 | 0.80 | 0.85 | 1.669 * |
| T3         | 0.64 | 1.92 | 2.92 | 1.14 * | 5.32 | 5.10 | 0.71 | 0.84 | 0.90 | 1.14 * |
| LSD        | 0.455 | 0.698 | 1.28 * | 0.478 | 0.309 | 0.341 | 0.328 | 0.276 | 0.281 |

Change in soluble nitrogen content of soluble nitrogen (SN) and non-protein nitrogen (NPN) during maturation.

Table 4 shows a SN ratio of 0.26% for T1, T2 and T3 respectively at the beginning of ripening. As the ripening period progresses, an increase in SN ratios was observed with the maturity period reaching 1.10, 0.981 and 0.930% for T1 T2 and T3 respectively, as well as non-protein NPN, whose values increased at the end of the maturation process for all the treatments ranging from 0.90 to 0.98% after ranging from 0.183-0.190% at the onset of ripening. The reason for this evolution in the percentages of SN The NPN is based on proteolytic enzymes isolated by the added primer bacteria as well as the rennet proteases used in the cheese industry, which have a significant role in producing protein degradation in mature cheeses, which is reflected in sensory characteristics such as tissue, texture, taste and flavor [32].

Table 4. Percentage of soluble nitrogen and non-protein nitrogen in the cheddar cheese coated with micro laminate of Cinnamon extract during the 6-month storage period at 10 °C ± 2 °.

| Treatment | Cheese age(month) | (%)NPN | (% NPN/ TN) | (%) SN | (%) Sn / TN |
|-----------|-------------------|--------|-------------|--------|-------------|
| T1        | 0                 | 0.19   | 4.39        | 0.26   | 6.01        |
|           | 3                 | 0.78   | 16.84       | 0.85   | 18.35       |
|           | 6                 | 0.98   | 20.28       | 1.10   | 22.77       |
| T2        | 0                 | 0.183  | 4.32        | 0.26   | 6.14        |
|           | 3                 | 0.802  | 17.06       | 0.87   | 18.51       |
|           | 6                 | 0.912  | 19.40       | 0.981  | 20.82       |
| T3        | 0                 | 0.183  | 4.24        | 0.27   | 6.26        |
Sensory Evaluation: Figure 8 shows images of the various cheese treatments taken at the beginning and end of the 6-month maturation period, indicating the possibility of using the casing supported by the students' extract in the cheese coating and preservation during the ripening period as well as the anaerobic conditions provided by this casing. Also, contribute to the prevention of growth of the mold on the surface of the cheese as a controlled aerobic microorganisms. This is confirmed by the scores given to all the sensory characteristics studied in Table 5, which included appearance, cohesion and adhesion, texture, taste and decay and molds.

|   |   |   |   |   |
|---|---|---|---|---|
|   | 0.750 | 16.37 | 0.845 | 18.44 |
| 6 | 0.900 | 19.14 | 0.930 | 19.78 |
| LSD | 0.492* | 4.198* | 0.558* | 5.416* |

*P<0.05
Figure 8. Macaroni coated envelope cheese manufactured at the beginning and end of the maturity period of 6 months.

Table 5. Results of Sensory Evaluation in the Coated Protein Coagulation Process for the Plant Extract of the Students During the Maturation Period of 6 Months and at 10 °C ± 2°

| Treatment | Cheese age (month) | Taste & flavor | Textures | Cohesion & adhesion | Appearance | Separation of fat | Growth of mould |
|-----------|--------------------|----------------|----------|---------------------|------------|------------------|-----------------|
| T1        | 1 day              | 9              | 10       | 9                   | 9          | 10               | 10              |
|           | 1                  | 9              | 10       | 9                   | 10         | 10               | 10              |
|           | 3                  | 8              | 9        | 9                   | 9          | 10               | 10              |
|           | 6                  | 6              | 7        | 5                   | 6          | 10               | 8               |
| T2        | 1 day              | 9              | 10       | 9                   | 9          | 10               | 10              |
|           | 1                  | 8              | 9        | 9                   | 10         | 10               | 10              |
|           | 3                  | 8              | 9        | 9                   | 8          | 10               | 10              |
|           | 6                  | 6              | 7        | 6                   | 6          | 10               | 10              |
| T3        | 1 day              | 9              | 9        | 10                  | 9          | 10               | 10              |
|           | 1                  | 9              | 9        | 8                   | 9          | 10               | 10              |
|           | 3                  | 8              | 9        | 8                   | 8          | 10               | 10              |
|           | 6                  | 8              | 8        | 8                   | 8          | 10               | 10              |
| LSD       | 3.07*              | 2.5            | 2.88     | 2.39                | 2.35*      | 2.27 NS          |                 |
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