Studying the Effect of Fortifying Edible Films Used to Coat Soft Cheese with Extracts of Thyme and Green Tea on the Chemical Properties of Soft Cheese

ABSTRACT

The study aims to manufacture edible gelatinous films with alcoholic extracts of thyme and green tea, to inhibit the E. coli growth as a negative bacteria and Staphylococcus aureus at a concentration of (0.100, 0.250, 0.500, 0.750, 1.000 mg), and determining the effect of this addition on the chemical tests of soft cheese stored for 14 days at a temperature of 7 ° C. The results indicate that the moisture percentage of cheese samples coated with gelatinous films decreased significantly (P <0.05) at the end of the storage period, and the treatment T2 gave the lowest percentage, which was 58.45% compared to the control sample T1 that was without gelatinous coating 58.13%. It was also found that the percentage of fat increased until the end of the storage period, and it gave significant differences at the level (P<0.05). As for the protein percentage, it does not give any significant differences at the end of the storage period. The pH starts to decrease and it gives significant differences to reach the end of the storage period for the treatments T2 6.17, T3 6.27 and T4 6.28 compared with the control sample T1 which reaches 5.96. It is also found that the ash percentage increased to the end of the storage period and gave significant differences, and the treatment T4 gives the lowest percentage of ash at (2.78%), while the percentage of carbohydrates occurs in a gradual difference between the treatments until the end of the storage period and does not give any significant difference at the level (P <0.05).

INTRODUCTION

A medicinal plant is one or more of its parts that contains one or more chemicals in a little or large concentration and can treat a specific disease or more or reduce the symptoms of infection if it relies on this plant part either in its natural form or by chemicals. The researcher Dragendorff explained in his definition of the medicinal plant that everything of plant origin is used medicinally. Many plants are added to the product (Parra, 2006), either in a fresh or dry form or extracts of those plants for the purpose of imparting the desired flavor to the product as well as for its inhibitory effectiveness towards the microorganisms and giving distinctive colors to the product to attract the consumer (Hayaloglu and Farkye 2011). Explained the use of many medicinal plants such as mint, thyme, students and nigella for the purpose of prolonging the storage period and reducing the number of microorganisms present in the cheese and giving a distinctive flavor to the cheese (Fadel, 2013). The plants were added in the form of aqueous extracts to the prepared milk for making cheese.

Thyme is one of the medicinal plants. It is an herbal plant that is used as a drink instead of tea or with tea and is added to some foods to give it an acceptable flavor (Delwing et al, 2016). The thyme plant has been used since ancient times to add flavor to cheese (Akarca et al, 2016).
As for green tea (Camellia sinensis), it is an antioxidant herbal plant with abundant health benefits and is also considered one of the most popular beverages in the world mostly due to its potential health care (Delwing et al., 2016). Green tea extract has recently been used as a natural additive to foods, especially cheeses (Senanayake, 2013).

**MATERIAL AND METHODS**

**Preparation of alcoholic extracts:**

The alcoholic extract of thyme and green tea was prepared according to the method mentioned (Chan et al., 2007) by weighing 100 gm of powder for each of the thyme and green tea vegetables were soaked in 250 ml of ethyl alcohol in a 500 ml volumetric flask. Then the mixture was stirred continuously for 24 hours, then filtered by using an eight-layer scourer cloth, the extract was centrifuged at 5,000 rpm for 10 minutes, and the resulting filtrate was collected in a glass flask. The solvent was then evaporated at room temperature to obtain the extracts for both plants. The mixture was filtered and centrifuged, then evaporated using a water bath at a temperature of 60 °C to obtain the extracted powder for both plants.

**Preparation of gelatinous films:**

The gelatinous films were prepared according to the method (Carvalho and Grosso, 2004) by weighing 10 gm of gelatin powder and dissolving it in 80 ml of distilled water. The solution was mixed for 5 minutes and stirred to the point of dissolution using a hot plate magnetic stirrer for 15 minutes, then heated at 60 °C for 15 minutes. Then add glycerol at a ratio of 3% of the weight of dry gelatin and complete to 100 ml by distilled water and adjust the pH to 7. Then the plant extracts at the required concentration were added to the solution prepared from the gelatinous membranes powder.

**Manufacture of Iraqi soft cheese:**

The soft cheese was made using the steps according to the method mentioned (Fox et al., 2017). The cow milk obtained from one of the milk suppliers in Salah El-Din Governorate was pasteurized at 63 °C for 30 minutes and after cooling to 45 ± 1 °C, rennet was added to it and left for a period 45 minutes until the stage cowardice was reached, after which the curd was cut to get rid of the whey and add 2.5% salt and put the curd in a damp cloth to get rid of the largest amount of whey. The curd was packed in special molds for each sample. Then the samples were labeled and kept in the refrigerator for microbial testing after 1, 7 and 14 days of storage.

**Coating soft cheese with films:**

The cheese samples were cut in a rectangular shape with a weight of 50 g for the sample to ensure that the cheese were completely contained and encapsulated with gelatin films. The treatments T2 were cheese treatment coated with gelatin and T3 cheese coated with gelatin and supported with alcoholic green tea extract, T4 cheese treatment coated with gelatin and supplemented with alcoholic thyme extract, while T1 was the control sample which was not coated, samples were left at a refrigerator temperature of 7 °C ± 1 until the coating hardened on the cheese surface after turning occasionally. Then it was stored by refrigeration at 7°C ±1 until tests were conducted on it and according to the suggested time period.

**Chemical tests of cheese:**

The percentage of moisture was estimated according to the method (Ling, 2008) and the percentage of protein and pH according to the method Hool et al. (2004). As for the percentage of fat, it followed the method mentioned by gerber (Min and Ellefson, 2010), and the ash was estimated in a method (A.O.A.C, 2004). The carbohydrate ratio was calculated mathematically according to the Ihekoronye method (1985) by the difference method.

\[
\% \text{ Carbohydrates} = 100 - \% (\text{ash} + \text{protein} + \text{fat} + \text{moisture}).
\]

**Statistical analysis:**

The data were analyzed statistically through the experiments system within the ready-made statistical program (SAS2012) and by using the completely randomize design system CRD. The averages were chosen according to the Duncan (Dancan, 1955) multi-term test to determine the significant differences between the averages of the factors affecting the studied traits at level of (P< 0.05).
RESULTS AND DISCUSSION

The chemical composition of thyme seeds and green tea:

In Table (1), the basic chemical composition of thyme seeds and green tea on the basis of dry weight, through which it can be seen that the moisture content of thyme seeds was 7.5% and green tea gave a relative Moisture of 7.1%, respectively. As for ash, the ash is a clear indication of the plant's content of Mineral elements, the higher the percentage of mineral elements, the higher the percentage of ash and vice versa. The percentage of ash reached 3.95%, which is higher than the percentage of ash in green tea seeds, which amounted to 3.15%, and this indicates the high content of plant seeds from the elements. The difference in the proportions of chemical components in plants may be attributed to the difference in the type of plant, and variety, harvest dates, environmental conditions, and geographical location (Aajina, 2006). It also contains a fat percentage of 33.85% in thyme and 28.35% in green tea, where fats are present in the seeds at a higher percentage than other parts of the plant. Because it constitutes food for the embryo in the seed, and thyme and green tea seeds contain protein percentage of 6.11% in thyme and green tea 14.72%, and thyme seeds contain a good percentage of protein, which may compete with some foodstuffs such as eggs 13.6% (in quantitative terms) and brown bread 8.7% and rice 6.7% (Pearson, 1971) The results indicated in the table that the seeds contain 48.59% carbohydrates in thyme and 46.68% in green tea.

Table (1) The chemical composition of thyme seeds and green tea

| Kinds of seeds | Moisture% | Ash% | Protein% | Fat% | Carbohydrates% |
|----------------|-----------|------|----------|------|----------------|
| Thyme          | 7.5       | 3.95 | 6.11     | 33.85| 48.59          |
| Green tea      | 7.1       | 3.15 | 14.72    | 28.35| 46.68          |

The chemical composition of soft cheese

Estimation of moisture content:

Table (2) indicates the moisture content of the soft cheese samples T1 uncoated treatment, While T2 treatment was the soft cheese samples coated with gelatinous Film only and the samples T3 and T4 treatments coated with gelatinous films supported with the alcoholic extracts green tea And thyme respectively. The results of the statistical analysis showed a significant decrease for the treatments at level of (P<0.05).) In the percentage of moisture during storage, which reached 14 days. On the first day of storage the moisture was (60.29, 60.05, 60.43, 60.43) % for T1, T2, T3 and T4 respectively. This result is considered among the Iraqi standard specifications (1988), which indicates that the moisture content in Soft cheese is not less than 50%.

Table (2) The effect of different treatments on the percentage of moisture in soft cheese coated with gelatin films

| Adjectives | Moisture% |
|------------|-----------|
| Treatments | 1day  | 7day  | 14days |
| T1         | 60.29  | 59.15 | 58.13  |
| T2         | 60.05  | 59.02 | 58.45  |
| T3         | 60.43  | 59.51 | 58.60  |
| T4         | 60.43  | 59.72 | 59.00  |
| Days average | 60.36  | 59.35 | 58.545 |

*±0.252 a
±0.322 b
±0.361 c
The numbers represent the rate ± the standard error. The letters similar to the control group mean that there are no significant differences. The letters different from the control mean the presence of significant differences. The mentioned numbers represent an average of three replicates per sample.

With the continuation of the storage process for the 14th day in a row, we note that the cheese samples which was coated and supported with alcoholic extracts coated had a slight loss of moisture and were reached 58.45% and 58.60% for T2 and T3 treatments and 59.0% for treatment T4, while the control sample T1 was at 58.13% as it gave the lowest moisture percentage, Bourtoom (2009) emphasized that the reason for the discrepancy in the low moisture content is due to the protein films that have demonstrated high efficiency in moisture reduction compared to the non-coated treatments as it contributes to retaining moisture and prevents its evaporation. And the physical and enzymatic increases the efficiency of the reservation. Cerqueira et al. (2009) also confirmed that the decrease in the moisture content was at 19.6% for coated cheese compared to non-coated cheese, which reached 23.4%.

**Fat:** The results listed in Table (3) indicate that the percentage of fat in control cheese, cheese coated with gelatinous films and cheese coated with gelatinous membranes supported with alcoholic extracts in treatments T1, T2, T3, T4 on the first day of storage was 16.03, 16.09, 16.40 and 16.33%, respectively. The reason for the discrepancy is the difference in the values of moisture content between the treatments (Ezzat and El-Shafei, 1991; El-Batawy et al, 1992; Lane and Fox, 1996).

### Table (3) The effect of different treatments on the percentage of fat in soft cheese coated with gelatin film

| Adjectives | Treatments | Fat%          |
|------------|------------|---------------|
|            |            | 1day | 7day | 14days     |
|            | T1         | 16.03 | 16.35 | 16.42       |
|            | T2         | 16.09 | 16.57 | 17.04       |
|            | T3         | 16.40 | 16.37 | 16.78       |
|            | T4         | 16.33 | 16.53 | 16.67       |
|            | Days average | 16.21±0.180 | 16.45±0.109 | 16.72±0.257 |

The numbers represent the rate ± the standard error. The letters similar to the control group mean that there are no significant differences. The letters different from the control mean the presence of significant differences. The mentioned numbers represent an average of three replicates per sample.

It is also noted from the table that the percentage of fat increases with the increased storage time, as the fat percentage was between 16.03% to 16.42% on the first day of storage until until 14 day of storage for treatment T1 and between 16.09% to 17.04% for T1 T2 treatment and between 16.40% to 16.78% for T3 treatment And between 16.33% and 16.67% for T4 treatment respectively, and it is mentioned that the reason for the increase in fat percentage with the storage of maturity is due to the decrease in moisture and thus the high percentage of fat (Ezzat and El-Shafei...
This increase in the percentage of fat is due to the action of lipolytic enzymes produced from the initiating bacteria and others, and these results are consistent with what was mentioned by Abdel baky et al, (1982 ). It is worth noting that the percentage of fat during the ripening period of cheeses coated with gelatinous coatings containing antimicrobial agents was higher compared to the treatment left without coating (T1 treatment), and this is due to the role of these coatings in retaining moisture and then providing a more suitable growth environment to the activity of the initiator bacteria.

**Protein:** The protein material is an important source for many changes in the organoleptic characteristics related to the flavor and texture of the cheese during the storage period, this is related to proteolysis and the percentage of protein during storage is affected by the proportions of other components, especially the moisture content (Henriques et al, 2013). The percentage of protein for T1, T2, T3, and T4 treatments at the first day of storage were reached 17.85, 17.97, 18.66, and 18.28%, respectively.

**Table (4) The effect of different treatments on the percentage of protein in soft cheese coated with gelatin films**

| Adjectives | Treatments | Protein% |
|------------|------------|----------|
|            | 1day       | 7day     | 14days   |
| T1         | 17.85      | 18.11    | 18.76    |
| T2         | 17.97      | 18.13    | 18.18    |
| T3         | 18.66      | 18.83    | 19.16    |
| T4         | 18.28      | 18.55    | 18.64    |
| Days average | 17.703 ±0.982 A | 17.897 ±0.977 a | 18.192 ±1.092 a |

| Adjectives | 25 days | 8 day | 2 day |
|------------|---------|-------|-------|
| T1         | 17.85   | 18.11 | 18.76 |
| T2         | 17.97   | 18.13 | 18.18 |
| T3         | 18.66   | 18.83 | 19.16 |
| T4         | 18.28   | 18.55 | 18.64 |
| Days average | 17.703 ±0.982 A | 17.897 ±0.977 a | 18.192 ±1.092 a |

The numbers represent the rate ± the standard error. The letters similar to the control group mean that there are no significant differences. The letters different from the control mean the presence of significant differences. The mentioned numbers represent an average of three replicates per sample.

This result was similar to what was found by (El-Batawy et al, 1991; El-Shazly et al, 1993; Shakeel-Ur-Rehman et al, 1998) of the difference in protein percentage between the treatments due to the difference in the moisture content values. While after 14 day of storage at 7°C the percentage of protein increased gradually for all treatments, as the percentage of it was for treatment T1 18.76%, for treatment T2 18.18%, for treatment T3 19.16% and for treatment T4 18.64%, respectively, this result is identical. What was indicated by (El-Soda et al, 1990; Ezzat and El-Shafei, 1991) of the increase in the percentage of protein with the advancement of the ripening of During storage period cheeses due to the decrease in the moisture ratios in the treatments with the progression of ripening, and this result is in agreement with his with what mention by Al-Sharaji (2002) that the increase in the decrease in the moisture content of the cheese during the storage period affects the state of equilibrium. The proportions of other ingredients, including protein, lead to high levels of these components.

**PH values:** The pH values mean the natural acidity and the advanced acidity resulting from the fermentation of lactose sugar and its conversion to lactic acid by the action of enzymes sourced
Lactic acid bacteria are the starter bacteria, and the acidity shares a balance with other compounds to give the cheese taste and flavor and thus affect the consumer acceptance of cheese (Fox and Wallace, 1997). It is noted from Table (5) a decrease in the pH of the all treatment of soft cheese produced in all, as the PH of T1 treatment was between decreased from 6.28 to 6.11, While in T2 from 6.75 to 6.30, in T3 from 6.66 to 6.44, and in T4 from 6.63 to 6.41. This decreased in pH values due to the transformation of the remaining lactose sugar in cheese to lactic acid during the first week and the second week of storage (Hofi et al, 1991; El-Shazly et al, 1993), while during storage process at day 14, the pH values of the treatments T1, T2, T3, and T4 decreased to 5.96, 6.17, 6.27, and 6.28, respectively (Fox and Wallace, 1997) indicated that most of the lactose (98%) in the milk was lost with the whey, and the fresh cheese contains on (0.7 - 1.5%) lactose is transformed mainly into lactic acid, which leads to a decrease in the pH of all types of cheese during the first weeks. We note that the changes in acidity of all coated and non-coated treatments depend on the relative humidity of storage that affects the activity of microorganism and these results indicate that gelatinous coatings indirectly contribute to the evolution of acidity by increasing the retention of higher moisture content to create a more favorable environment for bacterial activity (Ramos et al, 2012).

Table (5) The effect of different treatments on the pH values of gelatinous films

| Treatments | PH 1day | PH 7day | PH 14days |
|------------|---------|---------|-----------|
| T1         | 6.28    | 6.11    | 5.96      |
| T2         | 6.75    | 6.30    | 6.17      |
| T3         | 6.66    | 6.44    | 6.27      |
| T4         | 6.63    | 6.41    | 6.28      |
| Days average | 6.58 ±0.206 | 6.32 ±0.149 | 6.17 ±0.148 |

The numbers represent the rate ± the standard error. The letters similar to the control group mean that there are no significant differences. The letters different from the control mean the presence of significant differences. The mentioned numbers represent an average of three replicates per sample.

**Ash:** The term ash refers to the inorganic materials remaining after burning the organic matter in the food at a high temperature ranging between 500 and 600 °C. The minerals that make up these inorganic residues are present in the form of oxides, sulfates, phosphates, silicates and chlorides (called ion ash) and they depend on the composition of the food and the conditions of burning. As for the mineral compounds of ash, they include potassium, sodium, calcium, magnesium, a little bit of aluminum, iron, copper, zinc, and traces of lead and mercury found under special conditions (all of these are called cationic ash). The results in Table (6) indicate that the ash ratios for the T1, T2, T3, and T4 treatments at the first day of storage gave 2.01, 2.10, 2.75 and 2.80% respectively, the lowest ash percentage was for the treatment T1.
Table (6) The effect of different treatments on the percentage of ash in soft cheese coated with gelatin films

| Adjectives | Treatments | Ash% |
|------------|------------|------|
|            | 1day | 7day | 14days |
| T1         | 2.01 | 3.02 | 3.82 |
| T2         | 2.10 | 2.55 | 3.03 |
| T3         | 2.75 | 2.98 | 3.10 |
| T4         | 2.80 | 2.46 | 2.78 |
| Days average | 2.415 ±0.252 | 2.753 ±0.322 | 3.182 ±0.361 |

The numbers represent the rate ± the standard error. The letters similar to the control group mean that there are no significant differences. The letters different from the control mean the presence of significant differences. The mentioned numbers represent an average of three replicates per sample.

These results are in agreement with what Mahmoud, (2012) was found as he indicated that the percentage of ash increases during the storage period, and these results are in agreement with what El-zayat and Osman (2001) found, as we noticed also the ash content of cheese gradually increased with the progression of the storage period due to the decrease in the moisture content. After 14 days of storage ash percentage in treatment T1, T2, T3 and T4 was 3.82, 3.03, 3.18 and 3.78% respectively, the higher percentage in treatment T1 and the lowest in T4.

**Carbohydrates:** Carbohydrates are a major component in affecting the chemical properties of cheese, and they are considered energy sources in cheeses. The results in Table (7) indicated the percentage of carbohydrates for all treatments from the first day of storage until the 14th day, and they gave varying results, as the percentage of carbohydrates for the treatments T1, T2, T3, T4 were 3.82, 3.79, 1.76, 1.91% respectively. For the first day, up to the 14th day of storage, the treatment T2 gave the highest percentage, and it was at 3.30%. The results are consistent with what was mentioned (Aquileia et al, 2013) who have proven that the percentage of carbohydrates increases gradually the longer the storage period is prolonged. The reason for the gradual rise in the carbohydrate ratios of some treatments with the lengthening of the storage period can be attributed to the decrease in the moisture content ratios. The parameters can also be due to the difference in the percentage of moisture loss and the effect of the rest of the cheese components on the same carbohydrate percentage that was calculated mathematically.

The numbers represent the rate ± the standard error. The letters similar to the control group mean that there are no significant differences. The letters different from the control mean the presence of significant differences. The mentioned numbers represent an average of three replicates per sample.
Table (7) The effect of different treatments on the percentage of carbohydrates in soft cheese coated with gelatin films

| Adjectives Treatments | Carbohydrates% |
|-----------------------|----------------|
|                       | 1day | 7day | 14days |
| T1                    | 3.82 | 3.37 | 2.51   |
| T2                    | 3.79 | 3.73 | 3.30   |
| T3                    | 1.76 | 2.31 | 2.72   |
| T4                    | 1.91 | 2.75 | 2.91   |
| Days average          | 2.287±0.531 | 3.040±0.633 | 2.860±0.336 |

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دراسة تأثير تدعيم الأغلفة القابلة للأكل المستخدمة في تغليف الجبن الطري بمستخلصات الزعتر والشاي الأخضر على الخصائص الكيميائية للجبنة الطري

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الخلاصة
هدفت الدراسة الحالية إلى تصميم أغشية جيلاتينية قابلة للأكل مضاد لها مستخلص
كحولي من الزعتر والشاي الأخضر بتركيز ( 0.100, 0.250, 0.500, 0.750, 1.000) ملغ/ملغم (1) بهدف تثبيت نمو البكتيريا السالمة لصيغة كرام E. Coli بالإضافة إلى تحديد تأثير الزعتر والشاي الأخضر على الحفريات الكيميائية للجبنة الطري المخزن لمدة 14 يوم ودرجة حرارة 7 °C. وبناء النتيجة أن النسبة المئوية للرطوبة لعشاب الجبن المعقلة بالأغشية الجيلاتينية قد انخفضت معنويًا (P<0.05) عند نهاية فترة الخزنة. وقد أعطت المعاملة T2 أقل نسبة قد وكانت 58.45% مقارنة مع عينة السيطرة T1 التي كانت بدون غلاف جيلاتيني 58.13%. كذلك، وجد أن النسبة المئوية للدهون قد زادت إلى نهاية فترة الخزنة وقد أعطت فوائد معنوية عند مستوى (P<0.05) ووصلت على水平 سطح العلمية T2 إذ كانت 17.04% أما النسبة الضببية للبروتين فلم تعطى أي فوائد معنوية عند نهاية فترة الخزنة، أما قيم الUSD فينوني فقد بدأت بالانخفاض وأعطت فوائد معنوية لتصبح نهاية فترة الخزنة للمعاملات T2 وT3 هي 6.17 و6.27% على التوالي مقارنة مع عينة السيطرة T1 التي بلغت 5.96%. كذلك، وجد أن نسبة رماد إرادته إلى نهاية فترة الخزنة 14 يوم واعدة فوائد معنوية وان المعالمة T4 أعطت أقل نسبة رماد وكانت عند 2.78%، أما نسبة الكاربوهيدرات حصل فيها اختلاف تدريجي بين المعاملات إلى نهاية فترة الخزنة ولم تعطي أي فوائد معنوية عند مستوى (P<0.05).

الكلمات المفتاحية:
نبات الزعتر والشاي الأخضر، غشاء جيلاتيني قابلة للأكل، جبن طري، تركيب كيميائي.