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Evaluation of Alginate/Collagen Edible Coatings with Betanin and Cumin to Improve the Shelf Life of Lighvan Cheese

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ABSTRACT: Lighvan cheese is one of the most popular and consumed cheese in Iran. The aim of this study was to use coated alginates-collagen containing essential oils of cumin and betanin to improve the quality and shelf life of the Lighvan cheese. Initially, film samples containing different levels of cumin essential oils (4, 6, and 8%) and betanin (4 and 6%) were prepared, and then the physicochemical and antioxidant properties of all films were investigated. The physicochemical, antioxidant, and sensory properties of the samples of the Lighvan cheese were evaluated during 21day storage period. The results of experiments on edible films showed that the combination of essential oils of cumin and betanin into edible films increased the thickness, moisture content, water-solubility, and permeability to steam ($P<0.05$). The results indicated that the coated cheese samples containing cumin and betanin have effective antioxidant activity. Sensory evaluation results showed that the coated cheese samples had no negative effect on organoleptic acceptance ($P<0.05$). This study indicated that the addition of cumin essential oil and betanin to the alginate-collagen film had a positive effect on increasing the shelf-life of the Lighvan cheese. The selected sample contained essential oils of cumin and betanin at the concentrations of 8 and 6% respectively.

Keywords: *Alginate, Betanin, Cumin, Cheese, Collagen*.

Introduction

Cheese is an ancient food product that is made from different types of milk. Due to the significant increase in cheese consumption, its manufacturing industry is becoming a global business (Costa *et al*., 2018). Lighvan cheese is an Iranian semi-hard traditional cheese. It is produced from Ewe milk without a starter in the village of Lighvan (Ramezani *et al.,* 2017). In the process of its production, there is no heating process (pasteurization), and its microbial content may be due to the natural flora of milk and kimosin (Attar *et al*., 2017).

Generally, the complex combination of cheese and the conditions during transportation and storage often results in bacterial and fungal growth on the surface of the product. This leads to problems such as loss of quality and reduced shelf life of cheese, and economic losses to the cheese industry. It is expected that designing a suitable cheese coating can be one of the

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most effective strategies to mitigate the problems (Ramos *et al*., 2012).

Edible coatings are especially important in food storage because of their ability to improve the quality, food safety, moisture content, flavor, and aroma (Lopez-Rubio *et al*., 2017). Food films can be categorized into three types of proteins, polysaccharides, and lipids based on their main substance. Polysaccharides are widely available and inexpensive. Due to the large number of hydroxyl groups and other polar groups, the formation of hydrogen bonds in their structures plays an important role in the formation of these films. Examples of these are sodium alginate, pectin, and carboxymethyl cellulose (Dehghani *et al*., 2018) and from their protein types, collagen is the most abundant protein in the connective tissue of animals. Sodium alginate is a inexpensive and available bio-polymer that is obtained from seaweed. This natural material can form a strong film and performs better than other substances such as sodium caseinate and potato starch. Sodium alginate attracts particular attention due to its unique colloidal properties such as consistency, stability, gel production, water holding capacity, biodegradability, and biocompatibility. Sodium alginate is used to pack medications, bioactive molecules, proteins, and cells (Emamifar & Bavaisi, 2020).

Collagen is the main structural protein of the extracellular matrix found in the connective tissue of animals. The collagen is widely used in the food, pharmaceutical, and medical industries (Wang & Rhim., 2015; Wang *et al*., 2017). According to our knowledge, little research has been carried out on the use of a biologically active film such as sodium alginate and collagen with antimicrobial compounds to enhance the quality, longevity, and variety of cheese flavors. For example, Kooshki *et al*., (2015) examined the effect of calcium alginate edible coatings on microbial and chemical properties of Ewe cheese during storage in a refrigerator.

The present study is planned in two stages. Firstly, a qualitative and economical study was conducted based on biocompatibility using two biopolymers, alginates and collagen. In the second step, the purpose of the study is to add natural antimicrobial and antioxidant compounds to the variety of flavor and cheese colors, to improve the physicochemical properties, microbial properties and shelf-life of this dairy product.

In recent years, attention has been focused on the use of natural food additives as suitable alternatives to chemical additives. Also, the use of natural antimicrobial compounds in food packaging increases their longevity and maintains them by inhibiting the growth of microorganisms (Chen *et al*., 2014; Manahar *et al*., 2016). Natural colors are useful in improving the quality and increasing the nutritional value of food production. Consumers are interested in red color in foods and red beet is the best source of extraction. Betanin is a natural oral nitrogen pigment in red beet which is water-soluble and is the only bio-alkaline approved by the European Union for its use in food products. Betanin has a good redness for food and has several applications in foods such as gelatins, desserts, jams, and dairy products. (Ebrahimi & Shahriari, 2016). Researchers have found that betanin has bioactive properties such as antioxidant, antimicrobial, and anticancer activities (Manahar *et al*., 2016; Esatbeyoglu *et al*., 2014). The cumin essential oil has unique functional activities such as antibacterial, antifungal, anti-inflammatory, antiseptic, anticancer, anti-diabetes, and antioxidant properties. The antioxidant activity is mainly due to the presence of monoterpenes, flavonoids, and other polyphenolic compounds. Therefore, cumin is a potent antioxidant and antimicrobial agent in food preservation (Abbdellaoui *et* *al*., 2019).

In this study, a different scenario has been developed for the use of biocompatible films and natural additives. In the first step, the production of a inexpensive, natural film of alginate-collagen has become a priority. Secondly, the suitable natural additives that can increase the shelf life of the cheese were selected. The third step is the production of a type of cheese that has a different color, but the original flavor of the Lighvan cheese is preserved. Therefore, the effect of the Alginate-Collagen film and the effects of parameters such as different percentage of cumin (4, 6 and 8%) and betanin (4 and 6%) on the physicochemical and antioxidant properties of cheese stored at refrigerated temperature during different storage periods of 1, 7, 14 and 21 days were evaluated.

Materials and Methods

- *Materials*

Na-alginate (product number: 01- 37094) and collagen from the Kanto Chemical Company (Tokyo, Japan) and MSC Co. Ltd., (Sungnam, Gyunggido Korea) with analytical grade. Glycerol and Tween 80 (Fluka, Sigma Aldrich, MO, and USA) were used to provide film-forming dispersions (FFDs). The MRS broth and BHI (broth and agar) were purchased from Darmstadt, Germany. Foline Ciocalteu, sodium carbonate, standard gallic acid, and 2,2-diphenyl-1-perryl hydrazillin (DPPH) from Sigma Chemical Co (St. Louis, MO) was purchased. The essential oil was purchased from Sigma Aldrich (St. Louis, MO) and commercial betaine from Jena, Germany. Other materials include NaOH, Calcium Nitrate, Sodium, and Calcium Chloride from Merck (Whitehouse Station, NJ 08889-0100 USA).

- *Methods*

a. Preparation of film Alginate – Collagen

Combined Alginate-Collagen (A / C) film was prepared using solvent casting (Rhim & Wang., 2013). The film solution was prepared by dissolving 2 g of alginate powder and collagen (1: 1 ratio) into 100 ml of distilled water with 0.9 g of glycerol as a plasticizer and at 25 ° C for 95 minutes using a strong magnetic stirrer mixed. The temperature is controlled by a digital thermometer # 91000-055 / f. Controlled. The solutions of alginate-collagen film was prepared with essential oil of cumin (4, 6 and 8% wt%) and betanin (6, 8 wt%). Then, this solution was added to the combined Alginate-Collagen film. The samples were homogenised at 12,000 rpm by a homogenizer (IKA T25-Digital Ultra Turrax, Staufen, Germany) for 5 minutes. The emulsion was placed to remove air bubbles in a centrifuge (3000 rpm for 2 minutes). Solutions prepared for film production at 25 ± 2 °C were stored in a desiccator containing a saturated solution of 6H2O $Ca(NO_3)_2$ with a relative humidity of 50 ± 2% for at least 48 hours before the test.

In order to determine the characteristics of the desired coating, the sample solution was poured onto a Teflon-coated glass plate (24 cm × 30 cm) (Cole-Parmer Instrument Co., Chicago, Ill., USA) and dried at room temperature for 24 hours.

- *Specification of film features*

The thickness of the film was measured using a micrometer (Mitutoyo No. 293-766, Tokyo, Japan) with a precision of 0.001 mm. The moisture content of the films was determined according to Fajardo *et al*., (2010) in three replicates. Films were fixed in an oven at 105 °C to reach the weight. In order to calculate moisture, five repetitions of each of the films were used. Three random samples of each kind of film were placed in a 50 ml beaker containing 30 ml distilled water. The moisture content was determined by measuring weight loss at 105 C for 24 h until constant weight.

The solubility of film samples in water was measured by immersion in distilled water for 6 hours (Mei *et al*., 2013). The non-soluble dry matter was determined by removing the film pieces and then drying in an oven (105 ° C, 24 hours). The weight of water-soluble materials was calculated by removing the weight of non-soluble dry matter from the initial dry matter weight and the solubility percentage was calculated based on dry matter.

The Water Vapor Permeability (WVP) was tested using the gravimetric technique. Gravimetric techniques are commonly used to determine WVP of edible films. This test has been standardised by ASTM method (ASTM, 2005) method. In this test, the cups were filled with water and about 1.5 centimeters of air between the film surface and the water surface. The films were cut to the size of the cup mouth and held with the help of parafilm on the cup. Then, it was placed in a desiccator containing silica gel (relative humidity of zero percent). The samples were weighed every 2 hours. The permeation cells were weighed at regular time intervals until changes in the weight were recorded to be the nearest to 0.001 g. WVP was calculated as follows:

$$\mathrm{wvp} = \mathrm{m}\delta/\mathrm{At}\Delta\mathrm{p}$$

Where: m is the weight of water permeated through the film (g), δ is the thickness of the film (m), A is the permeation area (m^2), t is the time of permeation (s), and Δp is water vapor pressure difference across the film (Pa).

The antioxidant activity of the film was determined by the method of Haddar *et al*., (2012), and the percentage of free radicals inhibition by the steady-state radical 2, 2-diphenyl-1-perric acid hydrazyl (DPPH) samples. Twenty-five mg of each film was dissolved in 5 ml of distilled water, and then a tenth of a milliliter of the solution of the film was added to 3.9 ml of DPPH solution (0.1 ml of methanol solution) and continued for 60 minutes at ambient temperature and in Darkness was kept. The absorbance of the samples against pure methanol was read at 517 nm using a Perkin-Elmer spectrophotometer and the DPPH radical percentage was calculated by the following equation:

$$\text{The activity of trapping free radicals (\%)} = \left(\frac{\mathrm{ADPPH} - \mathrm{Asample}}{\mathrm{ADPPH}}\right) \times 100$$

The film surface color was measured using a Hunter Lip (Konica Minolta, CR-400, and Tokyo, Japan). A standard white-colored screen (L_0 = 97.49, a_0 = -0.09 and b_0 = 0.25) was used as a back pad for color measurement. Hunter's color indices (L*, a*, and b*) were calculated from five readings from each sample. The total color difference (ΔE) is calculated as follows:

$$\Delta E = \sqrt{(Lo - L^*) + (ao - a^*) + (bo - b^*)}$$

b. Cheese coverage by Coating

The surface of the Lighvan cheese cubes of 1 × 1 cm was coated with Alginate-Collagen oral solution (Immersion method). The cheeses were kept at controlled temperature and humidity (15 ° C, 70% relative humidity) for 5 hours until the coating completely dried. Covered cheeses were kept at 4 ° C for 21 days and were compared with uncoated samples.

- *Quality analysis of cheese*

The moisture content of the Lighvan cheese was determined by the gravimetric method for three hours at a temperature of 103 ± 2 ° C (Margolies & Barbano., 2018). Textures of samples were measured using a tissue parser (TA-CT3 Brookfield, USA). The hardness of the cheese was measured by the compression method with a 6 mm diameter cylindrical inverting probe. Test parameters: 1.0 mm/s before speed and after speed, 0.5 mm/s test speed, 50% distance and tear test 1.0%. The inner-outer hardness was determined at 3 ± 1 ºC with a penetrometer (4500 CT3 texture analyzer in Brookfield Made in USA) (Coffee & Coffee., 2016).

The color of the cheese surface was determined by a Hunter Lip (Konica Minolta, CR-400, Tokyo, Japan). Measurements are expressed as L (lightness), a (red / green), and b (yellow / blue). The color difference (ΔE) was calculated to the standard plate parameters, using the following equation:

$$\Delta E = \sqrt{(Lo - L^*) + (ao - a^*) + (bo - b^*)}$$

A standard white sheet (L_0 = 97.49, a_0 = -0.09 and b_0 = 0.25) was used as background for color measurement. Hunter's color indices (L*, a*, and b*) were calculated for each sample and mean value determined from five replications.

2-Thiobarbituric acid (TBA) (mg Malondialdehyde/kg) was determined according to Kirk and Sawyer (1991) method. Each reported TBA represents at least three repetitions for each sample.

Determination of antioxidant activity of cheese samples was carried out using DPPH free radical inhibitory method according to Haddar *et al*., (2012). For this purpose, 500 µl of the solution of each sample was mixed at concentrations of 3-5 mg/ml with 500 µl of ethanol 99.5%, and 125 µl of DPPH was added in 0.02 cc ethanol. Then, it was incubated for 60 minutes at room temperature and finally, the absorbance of the solutions was read at 517 nm. For control, all of the items mentioned above were applied except sample.

- *Sensory evaluation*

Evaluation of the sensory parameters of cheese was evaluated by a team of 15 trained panelists. The evaluators were not informed about the formulation and the samples were coded with 3-digit random numbers. Jury members scored points for sensitive properties such as color, odor, texture, and overall acceptance using the 5-point Hedonic method (1: very poor to 5: very good). Evaluation was performed under a white fluorescent light in a sensory lab.

- *Statistical analysis*

Data were analyzed statistically for each test. Analysis of variance (ANOVA) has been used to assess the importance of the difference between factors and levels. A comparison of meanings was carried out by Duncan's multiple range tests. Statistical analysis was performed using SPSS software version 21 (SPSS Inc., Chicago, IL).

Results and Discussion

- *Physical properties of films*

Table 1 presents the effect of betanin and cumin on the physical properties of alginate-collagen films. The thickness of the films containing betanin and cumin has increased ($p < 0.05$). The increase in the thickness of film samples by adding extracts and herbal essences is probably due to transverse ligaments that are created between film and phenolic compounds with a high molecular weight of the extract or essential oil. Due to its hydrophilicity and high molecular weight, phenolic compounds can reduce the effects of film softening and ultimately increase the thickness (Adams *et al*., 2005). According to Han & Krochta (1999), the film thickness is influenced by the solid content of the film-forming solution. Therefore, organic essential oils may cause loose film-forming network and increase the thickness.

Adding betanin to weight percentages of 4 and 6% increases the moisture content that results in the degradation of the film network, which increases the amount of water molecules that exist between the polymer chains by hydrogen bonding (Garcia *et al*., 2004). Although 4% cumin composition in films does not significantly affect water solubility, but by adding 6 and 8% cumin, the water solubility in alginate-collagen films increases as compared to the control sample. These findings are consistent with the data reported by Aguirre *et al*., (2013), which stated that this increase in soluble matter may be due to the low interaction of polymeric networks with the presence of essential oils.

Table 1 also shows the potential for water vapor penetration (WVP) films. All films containing betanin and essential oils of cumin significantly had higher WVP values than pure alginate-collagen films. Increasing the amount of water vapor permeability is probably due to the formation of an open and spongy structure due to the addition of essential oils of cumin and betanin. It appears that the sponge structure created in these films is due to the collapse of the regular structure of the polymer chains due to the presence of essential oils and betanin. This phenomenon increases the steam flow rate in the films.

- *Antioxidant activities of films*

The inhibition of free radical activity was determined by films with and without betanin/ cumin essential oils as shown in Figure 1. Alginate-collagen films showed inhibitory activity of 0.0% radical activity in DPPH. Films containing 8% cumin essential oil showed a greater effect on inhibiting radical activity with values of 75.68% and 74.60%. Antioxidant activity increased with increasing essential oil levels of cumin and betanin. The antioxidant power of the essential oil of the cumin is due to the presence of high amounts of Monoterpan, Linalool, Carvacrol, Karoon, Limonene, Anthol, Strugel, Flavonoids, and other polyphenolic compounds (Najda *et al*., 2008). Cumin is a potent antioxidant capable of scavenging hydroxy, peroxy, and DPPH free radicals and thus inhibits radical-mediated lipid peroxidation. The high antioxidant activity of cumin can be correlated to the high phenolic content. The antioxidant activity of cumin significantly correlated with the total phenol content of cumin extract. The phenol extract of cumin contained an array of phenolic compounds that may be responsible for its antioxidant activity. The total phenolic content of methanolic extracts of cumin varieties ranged from 4.1 to 53.6mgg–1 dry weight (Ani *et al*., 2006). Generally, the antioxidant activity of films is a function of the type and amount of antioxidant compounds used in the film and the inherent antioxidant properties of the film (Gomes Stacka *et al*., 2009).

Table 1. Physical properties of alginate-collagen films containing different percentages on Cumin and Betanin

Films	Thickness (mm)	Moisture (%)	Water Solubility (%)	Water vapor permeability ($\times10^{-6}$ g/m^2.s.Pa)
T_0	0.165±0.007^{c}	7.87±0.25^{b}	78.87±3.90^{b}	3.05±0.29^{d}
T_1	0.220±0.014^{bc}	7.95±0.65^{ab}	83.45±2.15^{ab}	3.80±0.26^{c}
T_2	0.250±0.022^{ab}	8.50±0.47^{ab}	85.70±1.27^{a}	4.70±0.28^{b}
T_3	0.210±0.028^{bc}	7.75±0.48^{ab}	84.40±3.53^{ab}	3.90±0.21^{c}
T_4	0.305±0.015^{a}	8.64±0.40^{a}	83.75±1.71^{ab}	5.75±0.38^{a}
T_5	0.215±0.021^{bc}	7.70±0.52^{b}	81.20±1.43^{ab}	4.05±0.23^{c}
T_6	0.307±0.021^{a}	8.57±0.48^{ab}	81.35±1.91^{ab}	5.25±0.30^{ab}

T_0= Control; T_1= 4%Cumin+4%Betanin; T_2= 4%Cumin+6%Betanin; T_3=6%Cumin+4%Betanin; T_4= 6%Cumin+6%Betanin; T_5= 8%Cumin+4%Betanin; T_6= 8%Cumin+6%Betanin

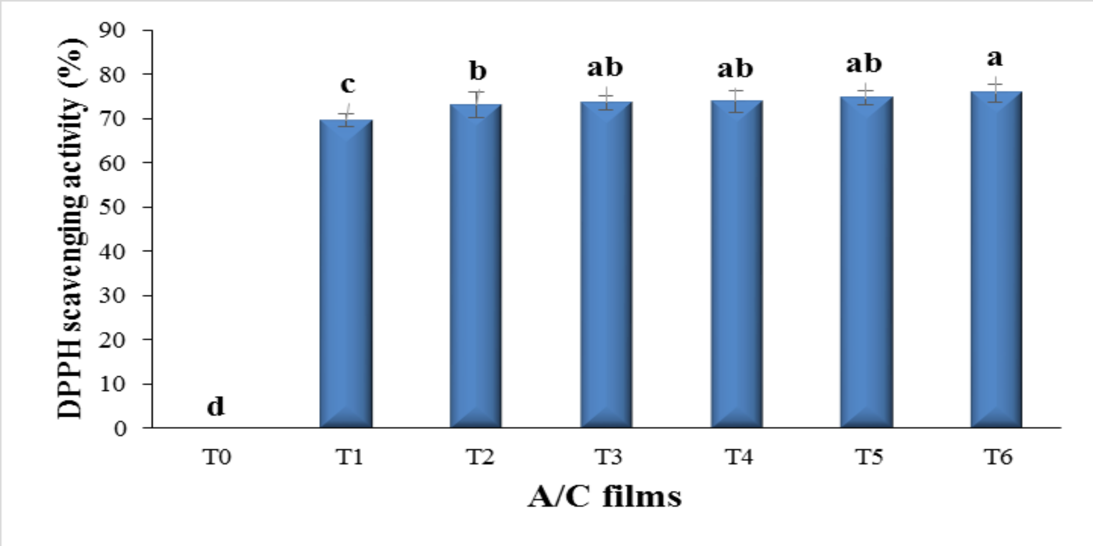

Fig. 1. Antioxidant activity of alginate-collagen films containing different percentages on Cumin and Betanin.

T_0= Control; T_1= 4%Cumin+4%Betanin; T_2= 4%Cumin+6%Betanin; T_3=6%Cumin+4%Betanin; T_4= 6%Cumin+6%Betanin; T_5= 8%Cumin+4%Betanin; T_6= 8%Cumin+6%Betanin

- Color indexes of films

In practical use, color affects the appearance of edible films, which in turn leads to consumer choice. Betanin composition in film formulation also increased the affected color of the films (Table 2). These results indicate the reddening of alginate-collagen films after the addition of the betanin to their formulation. These changes were due to the botanic red color. The combination of essential oil of cumin and film formulation also affected the color of the films and reduced the amount of L*. Also, Duo *et al*., (2011) reported that the addition of phenolic compounds to edible fillets made of apple puree made the films darker.

- Color indexes of cheeses

Pictures of the cheese samples were taken immediately after coating to check the distribution of the coating on the cheese (Figure 2). After forming the thin film, all levels of the cheese looked smooth and uniform. The results of this study showed that the packaging of Liquvan cheese samples with alginate-collagen film containing cumin and betanin essential oil, caused a significant decrease in color brightness and a significant increase in the values of a* and b* produced cheeses ($p < 0.05$). During storage, the color brightness of different cheeses was initially increased and then decreased. Zhong *et al*. examined

Table 2. Color indexes of alginate-collagen films containing different percentages on Cumin and Betanin

Films	L*	a*	b*	ΔE
T_0	87.91 ±0.34^a	-0.85 ±0.01^d	2.09 ±0.02^a	6.85 ±0.10^d
T_1	79.56 ±0.48^b	18.08 ±0.82^b	-1.00 ±0.01^e	23.52 ±0.80^b
T_2	79.11 ±0.80^b	18.78 ±0.50^b	1.02 ±0.02^c	23.56 ±0.71^b
T_3	78.94 ±0.68^b	13.31 ±0.58^c	1.24 ±0.06^b	20.41 ±0.67^c
T_4	76.56 ±0.87^c	18.43 ±0.92^b	-0.33 ±0.01^d	25.44 ±0.49^b
T_5	79.19 ±0.64^b	18.98 ±0.48^b	-1.42 ±0.03^d	24.24 ±0.41^b
T_6	75.34 ±0.49^c	23.08 ±0.81^a	-1.24 ±0.03^f	29.63 ±0.55^a

T_0= Control; T_1= 4%Cumin+4%Betanin; T_2= 4%Cumin+6%Betanin; T_3=6%Cumin+4%Betanin; T_4= 6%Cumin+6%Betanin; T_5= 8%Cumin+4%Betanin; T_6= 8%Cumin+6%Betanin the effect of edible coatings of chitosan, sodium alginate, and soy protein isolate on the quality properties of mozzarella cheese and observed that in coated cheese samples, the intensity of color brightness was higher than the non-coated samples. Sodium alginate coating increased the amount of a* mozzarella cheese samples. On the first day, the b* level of the sample covered with sodium alginate and soy protein isolate was significantly lower than the control sample (Zhong *et al.*, 2013).

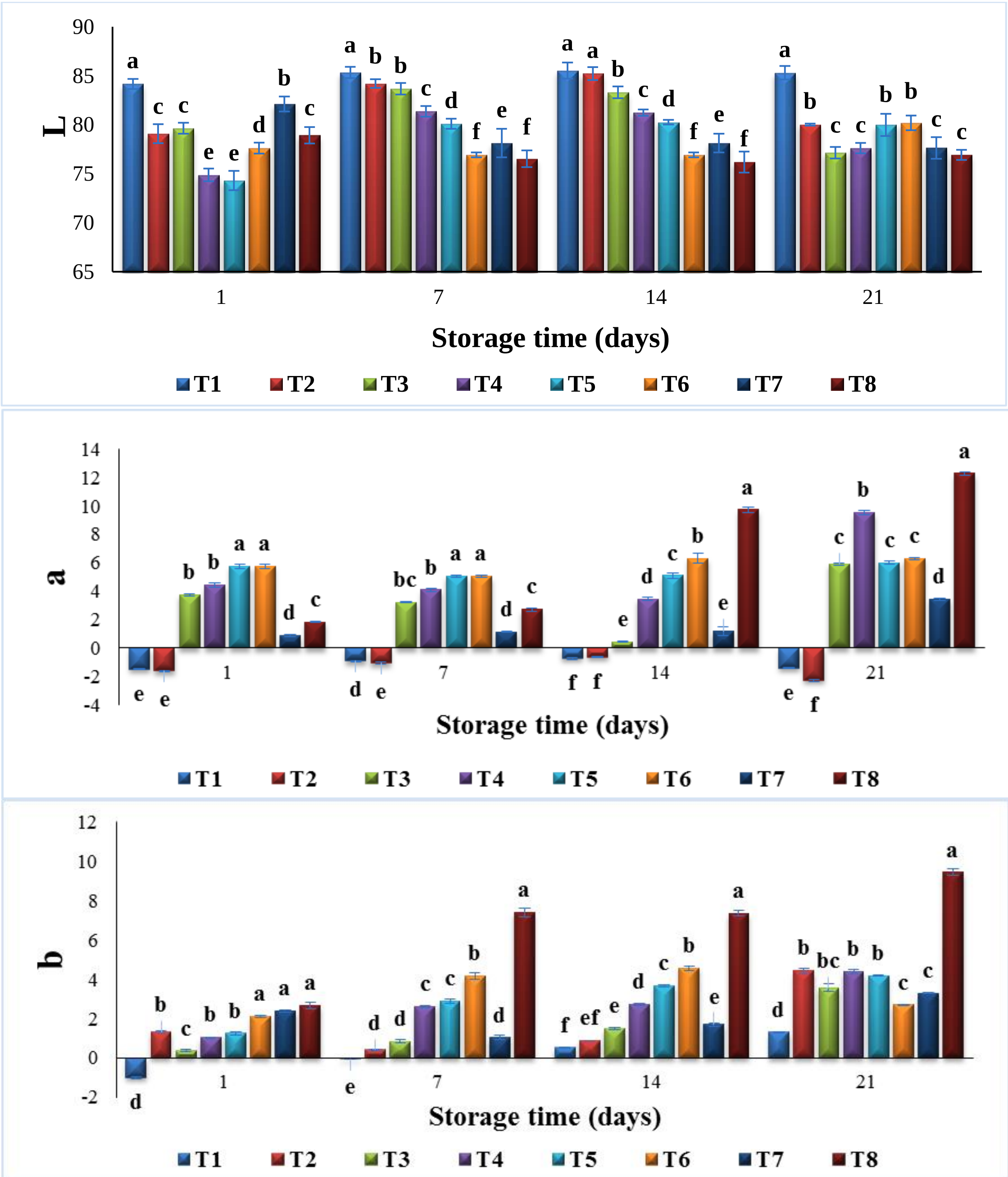

Fig. 2. Color indexes of Lighvan coated cheese during storage at 4^{o}C

T_1= Cheese without any coating (Control); T_2= coated cheese with collagen/alginate coating without cumin and betanin; T_3=Cheese coated by C/A film with 4%Cumin+4%Betanin; T_4= Cheese coated by C/A film with 4%Cumin+6%Betanin; T_5= Cheese coated by C/A film with 6%Cumin+4%Betanin; T_6= Cheese coated by C/A film with 6%Cumin+6%Betanin; T_7 = Cheese coated by C/A film with 8% Cumin+4% Betanin; $T_{8=}$ Cheese coated by C/A film with 8% Cumin+6% Betanin

- ***Moisture content***

According to the results reported in Table 3, in all the samples, moisture content decreased with storage time. In general, coated cheeses lost less moisture content than uncoated cheese during the shelf-life. During storage, the moisture content of all samples significantly decreased ($p < 0.05$), but its rate of decrease in the control sample and the coated sample (alginate-collagen) without essential oil of cumin and betanin were significantly higher than the other samples. Every day, the control sample showed the lowest amount of moisture. Increasing the level of essential oil of cumin in the treatments resulted in more moisture content in cheese samples. This was due to the reduced hydrophilicity of the coating (alginate-collagen) by the addition of the essential oil of cumin. Fajardo *et al.* showed that in cheese samples coated with chitosan, the moisture content is significantly higher than the control sample. During storage, the moisture content of the control sample and the sample containing chitosan coating decreased, but on all days, the coated cheese sample had a higher moisture content than the control sample (Fajardo *et al.*, 2010).

- ***Thiobarbituric acid index (TBA)***

Thiobarbituric acid (TBA) is an indicator of fat oxidation. It is widely used as an indicator for the evaluation of secondary oxidation products (Jeon *et al*., 2003). TBA changes in coated and uncoated cheeses during storage are presented in Table 4. The initial TBA was 0.015 mg MDA / kg for cheese samples. The amount of TBA of the control and samples coated during storage time increased and at the end of the maintenance period (day 21), the samples coated with solutions containing 8% essential oil of cumin and 6% of the betanin content showed significantly lower oxidation products. Perumalla & Hettiarachch (2011) showed that the antioxidant activity of EOs has been attributed to various mechanisms, including preventing the formation of a radical chain formation, a coupling of the metal catalyst, decomposition of peroxides, and interaction with free radicals. The essential oils of cumin and betanin have high antioxidant properties due to their high content of phenolic compounds (Kaur & Sharma., 2012; Escribano *et al*., 1998).

- ***DPPH Radical inhibitory activity***

Free radical control is one of the most well-known mechanisms by which antioxidant compounds can inhibit lipid oxidation. In Figure 3, the mean changes in the percentage of free radical inhibitory DPPH of different treatments of the Lighvan cheese during storage are presented. During

Table 3. Moisture content (%) of different cheese samples during storage periods

Samples	storage time			
	Day 1	**Day 7**	**Day 14**	**Day 21**
T_1	$54.04 \pm 1.43^{A,a}$	$38.88 \pm 1.33^{B,d}$	$29.04 \pm 1.52^{C,d}$	$22.95 \pm 1.01^{D,d}$
T_2	$54.79 \pm 1.0^{A,a}$	$40.79 \pm 1.80^{B,d}$	$32.02 \pm 1.03^{C,c}$	$25.04 \pm 0.84^{D,c}$
T_3	$54.62 \pm 1.71^{A,a}$	$44.95 \pm 0.84^{B,c}$	$36.83 \pm 0.88^{C,b}$	$30.22 \pm 1.83^{D,b}$
T_4	$54.28 \pm 1.36^{A,a}$	$47.94 \pm 0.98^{B,b}$	$36.80 \pm 1.00^{C,b}$	$31.39 \pm 1.39^{D,b}$
T_5	$55.02 \pm 1.01^{A,a}$	$48.49 \pm 1.28^{B,ab}$	$37.49 \pm 0.78^{C,b}$	$31.05 \pm 1.91^{D,b}$
T_6	$54.67 \pm 1.19^{A,a}$	$49.67 \pm 1.77^{A,ab}$	$37.63 \pm 0.67^{A,b}$	$30.18 \pm 1.21^{D,b}$
T_7	$54.29 \pm 1.86^{A,a}$	$50.62 \pm 1.29^{A,a}$	$39.61 \pm 0.59^{A,a}$	$34.13 \pm 0.97^{D,a}$
T_8	$56.21 \pm 1.64^{A,a}$	$50.41 \pm 1.97^{A,ab}$	$39.67 \pm 0.75^{A,a}$	$35.89 \pm 0.85^{D,a}$

T_1= Cheese without any coating (Control); T_2= coated cheese with collagen/alginate coating without cumin and betanin ; T_3=Cheese coated by C/A film with 4%Cumin+4%Betanin; T_4= Cheese coated by C/A film with 4%Cumin+6%Betanin; T_5= Cheese coated by C/A film with 6%Cumin+4%Betanin; T_6= Cheese coated by C/A film with 6%Cumin+6%Betanin; T_7 = Cheese coated by C/A film with 8% Cumin+4% Betanin; $T_{8\,=}$ Cheese coated by C/A film with 8% Cumin+6% Betanin the storage period, the antioxidant activity of the various cheese fluctuated, and in some days the trend was decreasing and in others, it was rising. In the last day of storage, the control sample (1.13 ± 0.23%) and the sample T8 (8.75% ± 0.07%) showed the lowest and highest percentage of activities respectively. Rafei *et al.* have reported the impact of antioxidant properties of cumin and tarragon essential oils on the quality of full-fat white cheese. They showed that cumin can delay the oxidation of cheese (Rafei *et al.*, 2017).

Table 4. TBA amounts (mg MDA/kg) of different cheese samples during storage periods

Samples	storage time			
	Day 1	Day 7	Day 14	Day 21
T_1	0.015 ±0.001^{A,a}	0.036 ±0.002^{B,a}	0.046 ±0.001^{C,a}	0.056 ±0.003^{D,a}
T_2	0.014 ±0.001^{A,a}	0.035 ±0.003^{B,a}	0.043 ±0.001^{C,b}	0.052 ±0.001^{D,a}
T_3	0.015 ±0.002^{A,a}	0.030 ±0.001^{B,b}	0.035 ±0.002^{C,c}	0.046 ±0.001^{D,b}
T_4	0.014 ±0.004^{A,a}	0.027 ±0.003^{B,bc}	0.034 ±0.003^{C,cd}	0.041 ±0.001^{D,c}
T_5	0.013 ±0.005^{A,a}	0.026 ±0.001^{B,c}	0.032 ±0.003^{C,cd}	0.038 ±0.001^{D,d}
T_6	0.015 ±0.001^{A,a}	0.025 ±0.004^{B,bcd}	0.028 ±0.004^{C,de}	0.035 ±0.002^{D,d}
T_7	0.015 ±0.002^{A,a}	0.024 ±0.003^{B,cd}	0.025 ±0.002^{C,e}	0.033 ±0.004^{D,de}
T_8	0.013 ±0.002^{A,a}	0.022 ±0.001^{B,d}	0.023 ±0.002^{C,e}	0.030 ±0.001^{D,e}

T_1= Cheese without any coating (Control); T_2= coated cheese with collagen/alginate coating without cumin and betanin; T_3=Cheese coated by C/A film with 4%Cumin+4%Betanin; T_4= Cheese coated by C/A film with 4%Cumin+6%Betanin; T_5= Cheese coated by C/A film with 6%Cumin+4%Betanin; T_6= Cheese coated by C/A film with 6%Cumin+6%Betanin; T_7 = Cheese coated by C/A film with 8% Cumin+4% Betanin; T_8 = Cheese coated by C/A film with 8% Cumin+6% Betanin

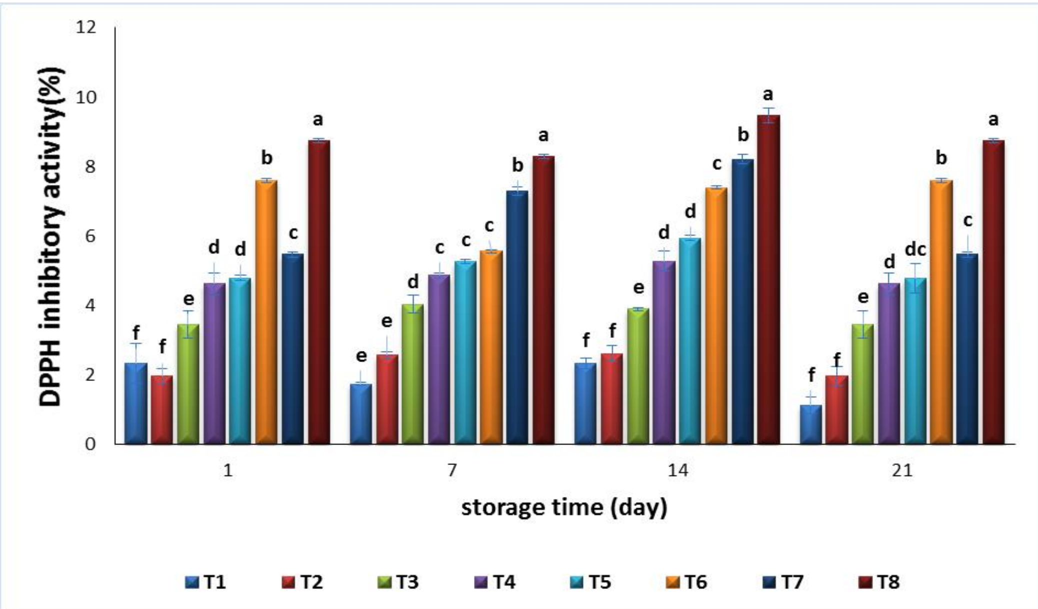

Fig. 3. DPPH inhibitory activity (%) of Lighvan coated cheese during storage at 4^0C

T_1= Cheese without any coating (Control); T_2= coated cheese with collagen/alginate coating without cumin and betanin; T_3=Cheese coated by C/A film with 4%Cumin+4%Betanin; T_4= Cheese coated by C/A film with 4%Cumin+6%Betanin; T_5= Cheese coated by C/A film with 6%Cumin+4%Betanin; T_6= Cheese coated by C/A film with 6%Cumin+6%Betanin; T_7 = Cheese coated by C/A film with 8% Cumin+4% Betanin; T_8 = Cheese coated by C/A film with 8% Cumin+6% Betanin

- *Hardness*

The hardness of the uncoated cheeses on the first day was (N.5.41) (Figure 4). The hardness of the cheese increased significantly over time ($P <0.05$) and increased to 98.98 N at the end of the storage period. In this study, the hardness of the Lighvan cheese increased with the process of losing moisture (Table 4).

In the first week, the hardness of coated cheese was lower than that of uncoated samples ($P <0.05$). Guerra-Martínez *et al*., (2012) stated that hydration of coated cheeses could help to reduce the hardness as compared to the control sample. It was also found that edible coatings generally reduced the process of increasing the hardness of the cheese during the maintenance period, that may be due to the capacity of water to be stored by them. In the present study, the hardness of coated cheese was roughly 14% to 65% less than that of uncoated cheese.

- *Sensory evaluation*

The results of the sensory analysis of cheese samples coated with the coating of alginate-collagen and uncoated (control) are presented in Figure 5. The main barrier to the use of essential oils, especially those that have a strong odor, as food preservatives is that they produce negative sensory effects when added to provide an antimicrobial effect. The combination of essential oils in the edible film matrix can be a solution to this problem. In this study, film-containing solutions of cumin essential oil had no negative effect on organoleptic acceptance of cheese samples evaluated by sensory evaluators ($p <0.05$). Other researchers have shown that the use of a small amount of cumin essential oil in cheese does not have a negative effect on the organoleptic acceptance. For example, Rafei et al., have reported the use of cumin essential oils on the organoleptic properties of full-fat white cheese. They stated that the use of high cocentration of herbal essential oils might affect the organoleptic properties of the product (Rafei *et al.*, 2017).

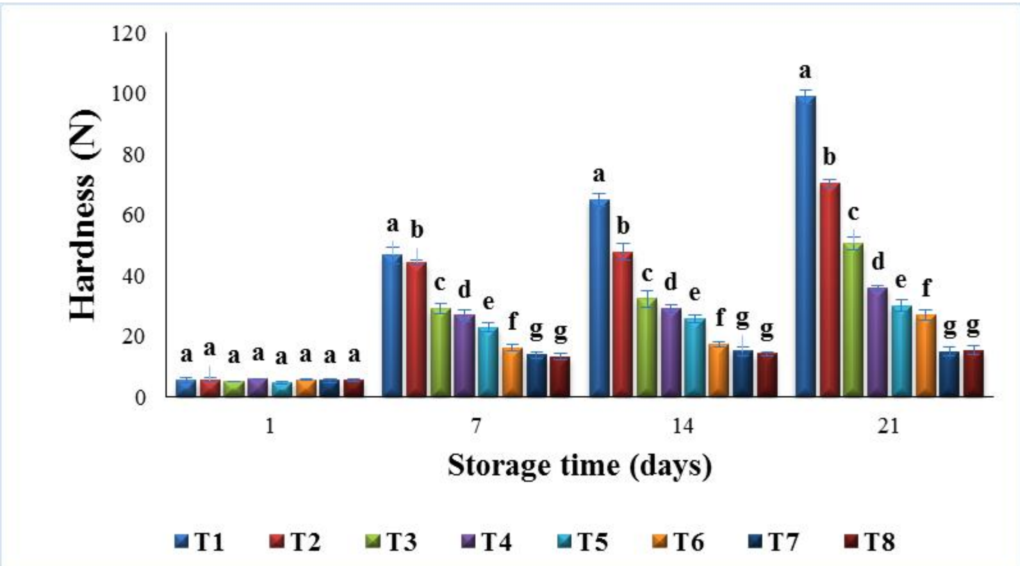

Fig. 4. Hardness amounts (N) of Lighvan coated cheese during storage at 4 ^{O}C

T_1= Cheese without any coating (Control); T_2= coated cheese with collagen/alginate coating without cumin and betanin; T_3=Cheese coated by C/A film with 4%Cumin+4%Betanin; T_4= Cheese coated by C/A film with 4%Cumin+6%Betanin; T_5= Cheese coated by C/A film with 6%Cumin+4%Betanin; T_6= Cheese coated by C/A film with 6%Cumin+6%Betanin; T_7 = Cheese coated by C/A film with 8% Cumin+4% Betanin; T_{8} = Cheese coated by C/A film with 8% Cumin+6% Betanin

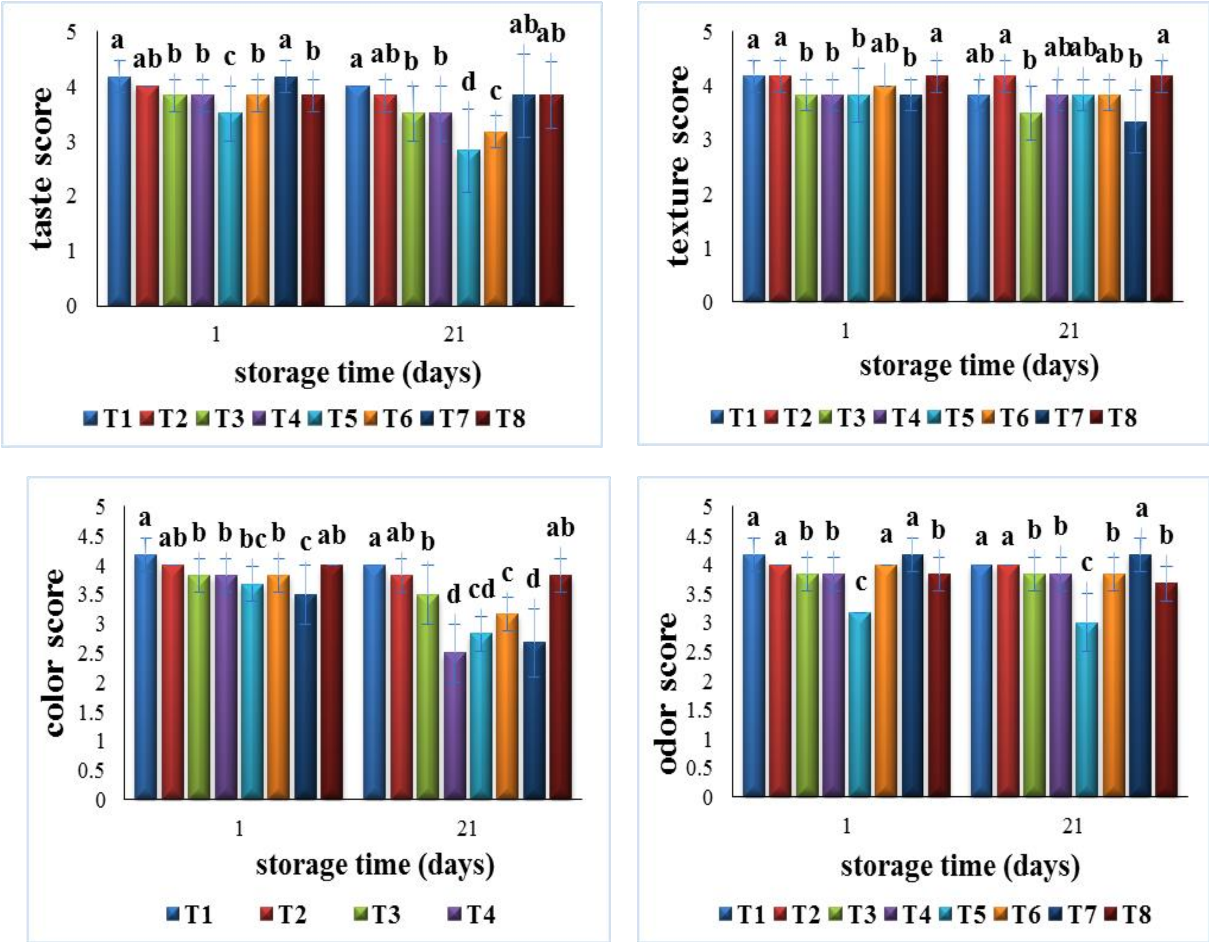

Fig. 5. Sensory characteristics of Lighvan coated cheese during storage at 4°C

T_1= Cheese without any coating (Control); T_2= coated cheese with collagen/alginate coating without cumin and betanin; T_3=Cheese coated by C/A film with 4%Cumin+4%Betanin; T_4= Cheese coated by C/A film with 4%Cumin+6%Betanin; T_5= Cheese coated by C/A film with 6%Cumin+4%Betanin; T_6= Cheese coated by C/A film with 6%Cumin+6%Betanin; T_7 = Cheese coated by C/A film with 8% Cumin+4% Betanin; T_{8} = Cheese coated by C/A film with 8% Cumin+6% Betanin

Conclusion

The results of this study showed that alginate-collagen films with cumin essential oils (4% to 8%) have high potential to be used in natural films to protect the food. By increasing the concentration of essential oil of cumin in films increased the permeability of water vapor, while solubility decreased. The results of this study showed that alginate-collagen films containing betanin and cumin essential oils, by preventing tissue changes and oxidation of lipids, increased the quality of the Lighvan cheese. Alginate-collagen films containing betanin and cumin essential oils had also antioxidation effect on the samples examined at the end of the storage period. The results showed that the effect of coatings containing 8% essential oil of cumin and 6% of betanin on the Lighvan cheese samples maintained their qualitative properties during storage, which was expressed by physicochemical and sensory evaluation results. Therefore, alginate-collagen film containing betanin and essential oils of cumin might be employed as an active coating to maintain the quality of the Lighvan cheese during storage at 4 °C.

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