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CHARACTERIZATION OF COMPISOTE EDIBLE FILMS FROM ALOE VERA GEL, BEESWAX AND CHITOSAN

Usman Amin, Muhammad Azam Khan, Muhammad Ehtasham Akram, Abdel Rahman Mohammad Said Al-Tawaha, Alexey Laishevtcev, Mohammad Ali Shariati

ABSTRACT
Environmental consciousness as well as individual's demand for ready to eat food, recently, has changed the trends in food packaging leading to the development of biodegradable and edible packaging. Emulsified edible films have better transparency, superior mechanical properties and provide barriers to water and other atmospheric gases. Edible films if not consumed, biodegrad chemically. In present study, edible films were, initially, prepared using Chitosan and *Aloe vera* at different concentrations. Films were then subjected to physical and mechanical testing. Films with 20% *Aloe vera* had low thickness as compared to films with no *Aloe vera.* These films also had superior mechanical properties and lower water vapor permeability. Films with 20% *Aloe vera* were, then, selected and beeswax was dispersed in Chitosan-*Aloe vera* solution at concentration upto 2.0% followed by film preparation through casting technique. Thickness and water vapor permeability were observed to be improved with increase in concentration of beeswax. Tensile strength of edible films was also improved 1.3 times when concentration of beeswax increased from 0.5 to 2.0%. Percentage elongation decreased with increase in beeswax concentration in the emulsified films. No change in particle size was observed with change in concentration of beeswax. Emulsions were also stable at room temperatures. Decrease in transparency of emulsified edible films was observed with increase in beeswax content in the emulsified films. In addition, cost analysis of the films proved them reasonable to be used as an alternate of synthetic packaging materials.

Keywords: edible film; chitosan; beeswax; *Aloe vera* gel; physiochemical properties

INTRODUCTION

Today, increasing the awareness of consumers about natural based food products with no chemical preservatives has led to explore innovative methods in preserving food to extend shelf life. The number of papers devoted to the developing of storage methodologies are on an increased and amongst them, edible coating has drawn considerable attention in recent years due to accentuated application of edible material in packaging over chemical types. In the technology of edible coating, the surface of food commodity is commonly covered by a thin layer of edible material; acting as a barrier to confine the gaseous movements and moisture transfer, control respiratory rate, and thereby reduce weight loss during storage **(Sogvar, Koushesh Saba and Emamifar, 2016)**. Moreover, a coat forming agent such as *Aloe vera* might have natural antioxidant and antimicrobial properties **(Vieira et al., 2016)** or be the carrier of those **(Koushesh Saba and Sogvar, 2016)**.

Aloe vera is an evergreen perennial plant belonging to the family *Liliaceae* and indigenous in countries with arid climate like Arabian countries. The reason to survive in tropical areas can be referred to its stems with high capacity of retaining moisture and its fleshy leaves **(Misir, Brishti and Hoque, 2014)**. Being an herbal medicator extensively consumed across the globe, *Aloe vera* has represented a long history of functional and remedial activities since ancient times through its broad spectrum of bioactive components **(Guo and Mei, 2016; Pothuraju, et al., 2015)**.

Besides myriad health benefits of *Aloe vera*, recently, it is being valorized by different pharmacy and food industries **(Soltanizadeh and Ghiasi-Esfahani, 2015)**. Technically, *Aloe vera* creates a gel which is found to be an antifungal agent, keeping effectively quality and inhibiting microbial spoilage through its antimicrobial activities **(Ahmed, Singh and Khan, 2009)**. In addition, *Aloe vera* gel has been shown to postpone oxidative browning, maintain moisture and control respiratory rate either in pre or post-harvest stages in a group of agro-food products like table grape **(Valverde et al., 2005)**, strawberry **(Vahdat, Ghazvini and Ghasemnezhad, 2010)**.

On the other hand, application of other natural bio-polymers like chitosan along with *Aloe vera* gel in coating surface of agricultural and crop products can enhance the gel functionality and synergize its above stated physiochemical properties.

Chitosan itself is renown to be used separately as edible coating in terms of its intrinsic features like being non-detrimental to human body, being environmentally friendly, possessing cationic nature and antimicrobial potency **(Alves and Mano, 2008; Pillai, Paul and Sharma, 2009)**. Beeswax, another natural compound, is also applied as coating agent. Beeswax consist of a long chain of aliphatic alcohols (oleate esters) with a possible chemical formula of $C_{15}H_{31}COOC_{30}H_{61}$. Beeswax in combination with chitosan, provides barrier to moisture and strength to the film. Food products have high moisture content and readily lose moisture after coming into contact with ecological conditions **(Velickova et al., 2013a)**. Nowadays, combination of different edible biopolymers to make a new edible coating is of much interest. Therefore, the aim of this study is to evaluate physio-chemically the edible film made from the combination of beeswax, chitosan and *Aloe vera* gel.

Scientific hypothesis

Edible films were developed using Aloe vera and chitosan and emulsified with beeswax using tween20 as an emulsifier.

The following hypothesis were tested: either beeswax had any effect on the the film properties or not. Increasing the concetration of beeswax in the films forming solution of Aloe vera and chitosan may improve the physical properties (thickness, moisture) of edible films. Increasing the concetration of beeswax in the films forming solution of Aloe vera and chitosan may improve the optical properties (transparency) of edible films. Increasing the concetration of beeswax in the films forming solution of Aloe vera and chitosan may improve the mechanical properties (tensile strength and % elongation) of edible films. Increasing the concetration of beeswax in the films forming solution of Aloe vera and chitosan may improve the barrier properties (water vapor permeability) of edible films.

MATERIALS AND METHODS

Materials

The research was conducted in different laboratories of Department of Food Engineering, University of Agriculture, Faisalabad. Chitosan, beeswax, glycerol and Tween20 was purchased from a registered scientific store at Faisalabad. *Aloe vera* was obtained from Department of Forestry and Range Management (FRM).

Preparation of *Aloe vera* gel

Aloe vera leaves, obtained from Department of Forestry and Range Management was stored at 8 °C from the time of harvest until utilization. Aloe vera then, washed using distilled water at 40 °C to remove impurities such as dust and dirt **(Pinzon, Garcia and Villa, 2018)**. After this, *Aloe vera* was peeled using ordinary knife in such a way that essential *Aloe vera* gel could be easily extracted. Then, *Aloe vera* gel was extracted in a glass beaker and blended using domestic blender resulting in a uniform solution. The solution was further screened by passing through sieves (0.1mm) to remove impurities. Pure *Aloe vera* gel solution was stored at 5 °C in a refrigerator to avoid the oxidation of phenol contents till further use.

Development of film forming solution

Acetic acid solution (2% v/v) was prepared by dissolving 2 mL of acetic acid in distilled water to make its volume up to 100 mL. Chitosan (2 g) was dissolved in 100 mL of (2% v/v) acetic acid solution to form 2% (w/v) chitosan solution. Films were prepared by varying the concentration of *Aloe vera* in chitosan solution from 0 – 20% as shown in Figure 1and Table 1.

Glycerol was added in the solution as a plasticizer. Then, films were developed by casting method by pouting the 20 mL of sample solution in the petri dishes (9 cm diameter). These petri dishes were placed in the desiccators at 40 °C for drying.

Development of emulsified edible films

After mechanically and physically testing the films, a suitable concentration of film forming solution was selected and emulsified with beeswax. For complete dissolution of beeswax, 0.25 mL of glycerol was added as a plasticizer and Tween20 (25% of oil) was added as an emulsifier. For preparation of emulsified films, selected Chitosan-*Aloe vera* solution was homogenized with beeswax (melted at 65 °C) at concentrations shown in Table 2.

To develop films, chitosan blended *Aloe vera* solution was heated up to 75 °C so that it can be mixed easily with beeswax. These films were homogenized at 13500 rmp for 1 min to develop the stable emulsions. This resulted in bubble formation in the film forming solution. These solutions were placed in the sonicator (ELMA E60H) to remove bubbles for 20 min. The film forming solutions were poured into petri dishes (9 cm diameter) in such a way to form a film of uniform thickness. These Petri dishes were shifted to the hot air oven/desiccators with the temperature of about 40 °C for the time until emulsified edible films were easily peeled off from the surface of petri dishes.

Evaluation of physic-chemical properties of film

Stability of emulsions

Stability of emulsions was determined according to **Purwanti et al. (2018)** with slight modifications. This was done by keeping the emulsion sample for 3 days to check the separation of beeswax due to Ostwald ripening or coalescence. A sample of 6 mL was taken and poured in a test tube. Tubes were placed in test tube rack at room temperature for 3 days. The initial height and final height was checked after 3 days to measure any oil separation. The emulsion stability was determined using following formula:

$$S = \frac{h0-ht}{h0} \times 100 \quad (1)$$

Where, S is the stability of emulsion, h_0 is the initial height of emulsion in the glass vial (cm), and h_t is the height of emulsion at the measuring time (cm).

Moisture content (MC)

The moisture content of edible films was calculated using oven drying method by drying in an oven (BOHA-102 Canada) at 100 ±2 °C till constant weight is reached. Initially, all the films were standardized at room conditions

(25 °C and 70% RH). Then, films were weighed and Moisture content was determined as "the percentage of weight loss in the initial weight, using:

$$MC(\%) = \frac{Mi-Md}{Mi-Mp} \times 100 \qquad (2)$$

Where, M_i is the mass of the film specimen and petri dish before drying (g), M_p is the mass of petri dish (g) and M_d is the mass of film specimen and petri dish after drying (g).

Thickness measurements

Film thickness was measured using Digital Micrometer (Model: Mitutoyo LC = 0.001 and Range: 0 – 25 mm, Japan). Firstly, the instrument was calibrated to check the zero error. Films were, then, peeled off from the surface of petri dish and thickness of the films was measured at four random positions along rectangular strip and at center. Average of all five values was taken as the film thickness in milimeters.

Transparency

Transparency of the films was measured using UV Visible Spectrophotometer (T80, UK). Firstly, films were cut down into uniform strips of cuvette size. These films then placed on one side of the cuvette with film side facing the lense. Wavelength of UV Visible was adjusted to 600 nm and calibrated with distilled water. Cuvettes were, then, placed into the jacket of UV Visible Spectrophotometer. Film's transparency was compared with transparency of distilled water. As the light passed through the films, % transmittance was displayed on software display panel.

Water vapor permeability

The water vapor permeability was determined by gravimetric analysis with ASTM E96-95 standard usually known as water vapor transmission rate (WVTR). Films were cut into a uniform size of 7x7 cm and standardized with ambient environment (25 °C and 70% RH). 10 mL of water was taken into the test tubes of 1.5 cm in diameter and 12 cm in height. Further, strength at the mouth of test tubes was provided with gum so that water cannot evaporate through openings. Then, these test tubes were placed in the desiccators at room temperature and 0% RH for 12 h. Relative humidity was maintained at 0% by using silica gel placed at different locations in the desiccators. Atmospheric pressure was calculated using barometer at 0% RH and at 100% RH. Mass lost during 12 h was measured during weight balance.

$$\text{WVP}(\text{mgm}^{-2}.\text{h}^{-1}.\text{Pa}^{-1}.\text{mm}) = \frac{\Delta m \times X}{A \times t \times \Delta P} \qquad (3)$$

Where Δm is the mass change over time (mg), X is the thickness (mm) t is the time (h), A is film area (m^2) and ΔP is the partial vapor pressure (Pa) difference of the atmosphere with silica gel and pure water (3179 Pa, at 22 °C).

Particle size analysis

Size of the particles of the emulsion was determined by Particle size analyzer (Better Size ST A8311, China). It describes the distribution of beeswax particles in the film forming solution of chitosan with minimum and maximum size. The sample cup was first auto-cleaned to remove all the contamination and impurities in the distilled. Emulsified beeswax in film forming Chitosan-Aloe vera blend was poured in the sample cup when all the sample the bubbles were removed from the water. The sample was poured in the cup until a constant absolute value reached between 5 and 10. After few minutes, machine showed the results in cumulatively as well as differentially. The particle size of 50% beeswax was checked at D50 which represents maximum particle size of 50% grains in the solution.

Evaluation of mechanical properties of films

Ultimate tensile strength (UTS) and percent elongation (% E) of the films was determined using a universal testing machine. Films were cut into a uniform size of 8 cm length and 3 cm in thickness was placed between the jaws of the machine.

Statistic analysis

Analysis of Varience (ANOVA) was applied using SAS 2.0 software and significance of means was tested at $p < 0.05$.

RESULTS AND DISCUSSION

Films thickness

Thickness of chitosan-based and emulsified films was observed to be significantly affected by the concentration of *aloe vera* and beeswax respectively at 5% probability ($p < 0.05$). Thickness of chitosan-based films was observed to be decreasing 74.1 μm at S1 to 52 μm at S5 with the increase in Aloe vera concentration in chitosan-based film forming solution. Reduction in film thickness was similar to the studies of **Khoshgozaran-Abras et al. (2012)** and emulsification of selected concentration of *Aloe vera* **(Abugoch et al., 2011)**. *Aloe vera* and chitosan with beeswax from 0% to 2.0% resulted in a slight increase in the thickness of the films at S52 but continuous reduction in the film thickness was observed from 131 μm at S52 to 100 μm at S54 (Figure 2). Film thickness reduced to 23.6% as concentration was changed from S52 to S55 which is opposite to the studies of **Velickova (2013b)** and **Indriyati (2018).**

Moisture content

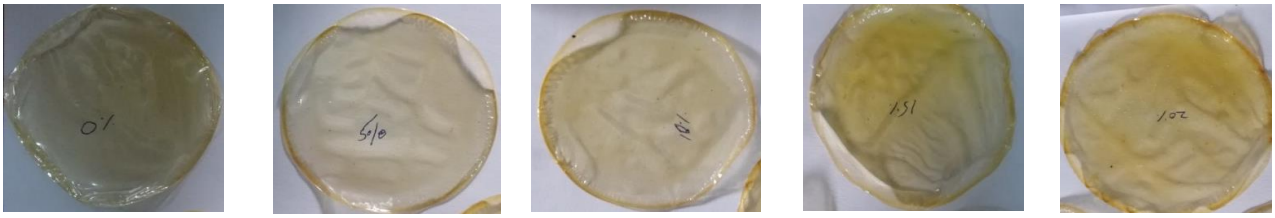
Films were subjected to environmental condition to check how much water these films can absorb. After standardization with environmental conditions, the moisture content was determined by oven drying method. Films of *Aloe vera* and chitosan observed to be significantly affected the moisture in the films ($p < 0.05$). Moisture content was continuously decreasing with increase in *Aloe vera* concentration. Maximum moisture content was observed at S1 (30.633%) which was observed to be continuously decreasing with increase in *Aloe vera* concentration at S5 (13.8667%). Emulsified *Aloe vera*-chitosan composite films were also treated with same protocol. The same effect was observed with increase in beeswax concentration 21.67% at S52 to11.86% at S55 in the emulsified films (Figure 3).

Table 1 Formulations of primary films.

Treatments	2% Chitosan Solution (%)	*Aloe vera* Gel (%)
S1	100	0
S2	95	5
S3	90	10
S4	85	15
S5	80	20

Table 2 Concentrations of emulsion using Beeswax.

Treatments	2% Chitosan Solution and *Aloe vera* (%)	Beeswax (%)
S51	100	0
S52	95	5
S53	90	10
S54	85	15
S55	80	20



S1 **S2** **S3** **S4** **S5**

Figure 1 Films of different concentration of *Aloe vera* (%).

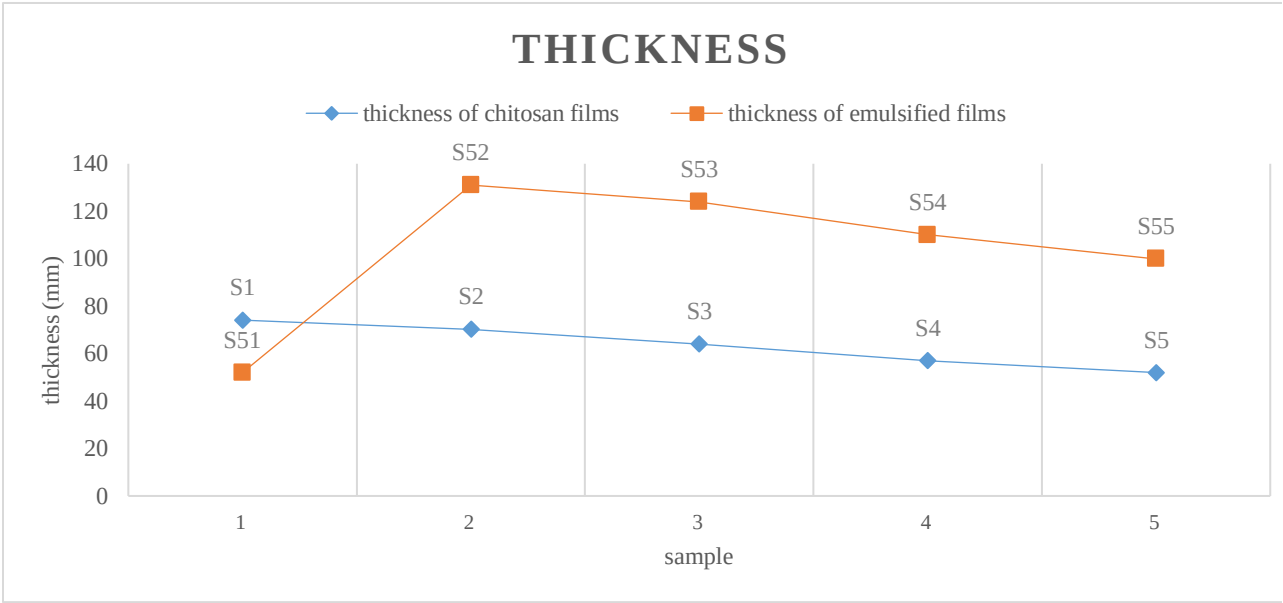


Figure 2 Comparison of thickness of chitosan and emulsified films. Note: *Si = Aloe vera concentration; S5i = Beeswax concentration; S1 = 0%; S2 = 5%; S3 = 10%; S4 = 15%; S = 20%; S51 = 0%; S52= 0.5%; S53 = 1.0%; S54 = 1.5%; S55 = 2.0%.

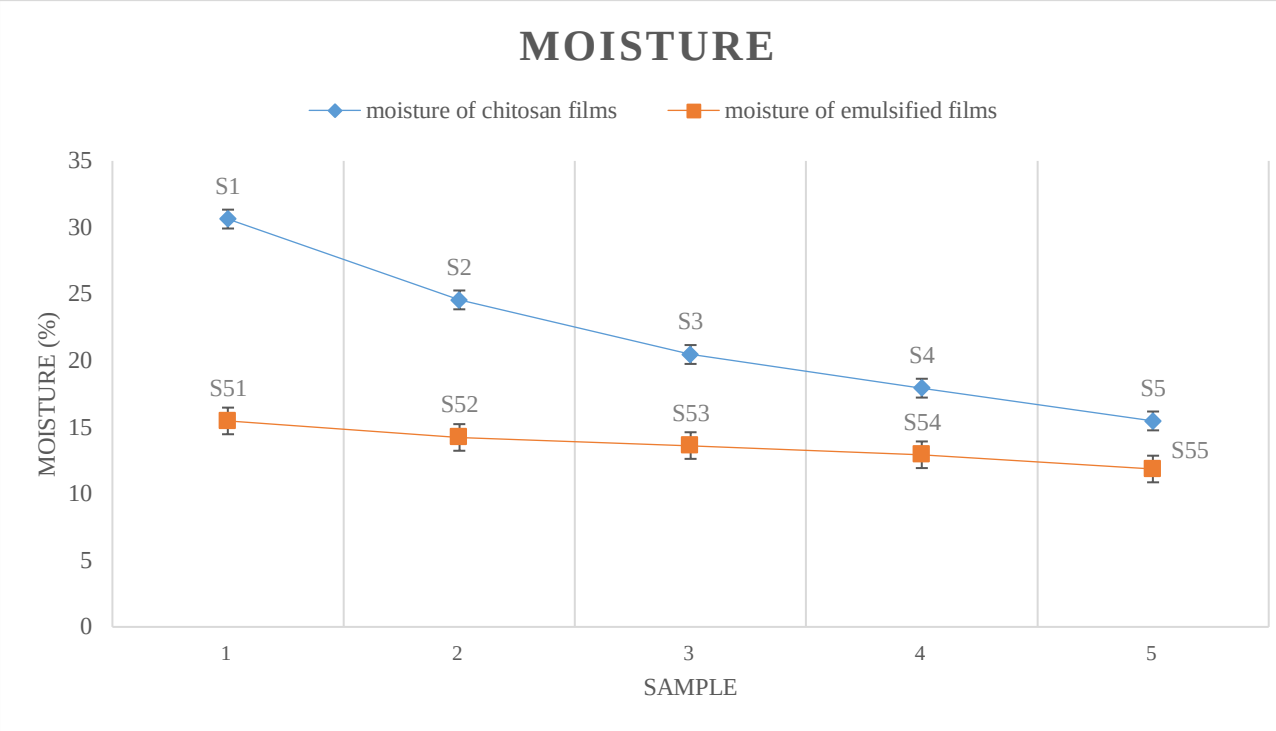


Figure 3 Comparison of moisture in chitosan and emulsified edible films. Note: *Si = Aloe vera concentration; S5i = Beeswax concentration; S1 = 0%; S2 = 5%; S3 = 10%; S4 = 15%; S = 20%; S51 = 0%; S52 = 0.5%; S53 = 1.0%; S54 = 1.5%; S55 = 2.0%.

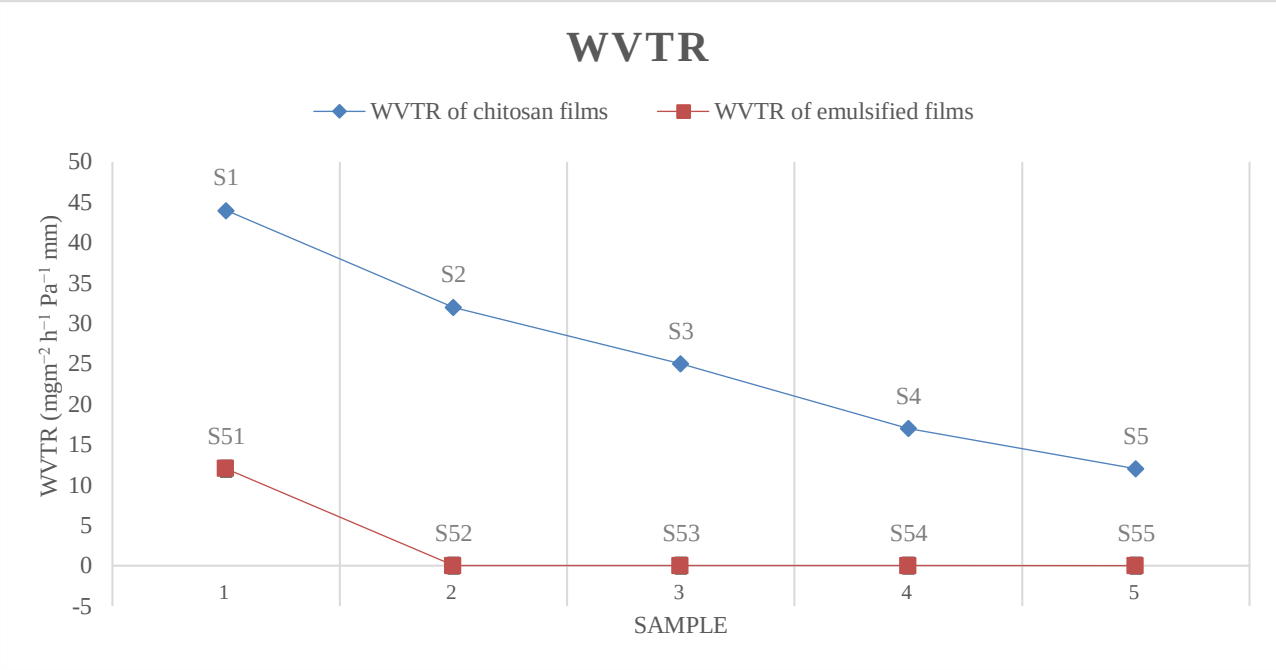


Figure 4 Comparison of water vapor permeability of chitosan and emulsified edible films. Note: *Si = Aloe vera concentration; S5i = Beeswax concentration; S1 = 0%; S2 = 5%; S3 = 10%; S4 = 15%; S = 20%; S51 = 0%; S52 = 0.5%; S53 = 1.0%; S54 = 1.5%; S55 = 2.0%.

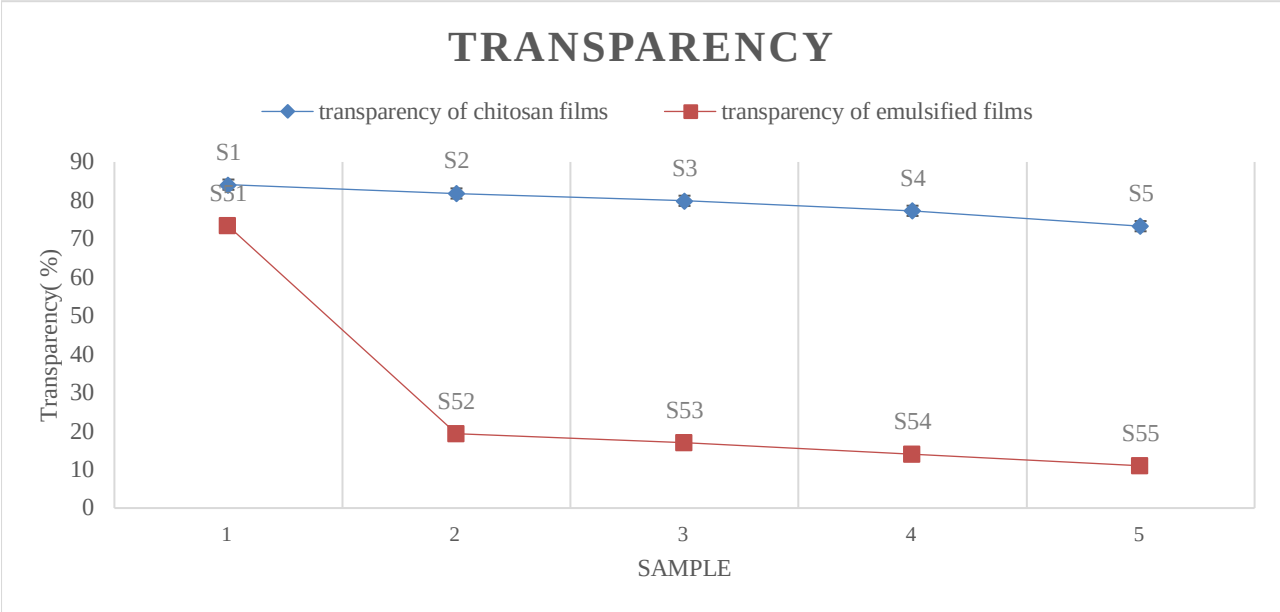


Figure 5 Comparison of transparency of chitosan and emulsified edible films. Note: *Si = Aloe vera concentration; S1i = Beeswax concentration; S1 = 0%; S2 = 5%; S3 = 10%; S4 = 15%; S = 20%; S51 = 0%; S52 = 0.5%; S53 = 1.0%; S54 = 1.5%; S55 = 2.0%.

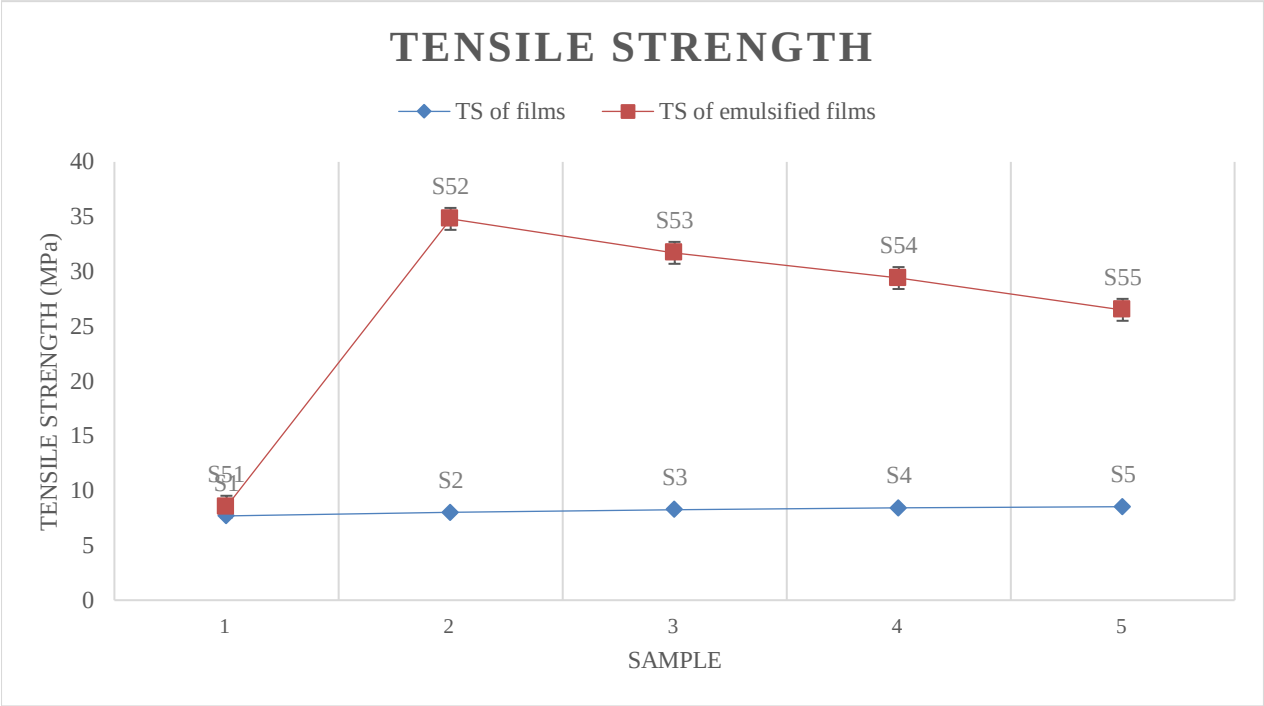


Figure 6 Comparison of tensile strength (TS) of chitosan and emulsified edible films. Note: *Si = Aloe vera concentration; S1i = Beeswax concentration; S1 = 0%; S2 = 5%; S3 = 10%; S4 = 15%; S = 20%; S51 = 0%; S52 = 0.5%; S53 = 1.0%; S54 = 1.5%; S55 = 2.0%.

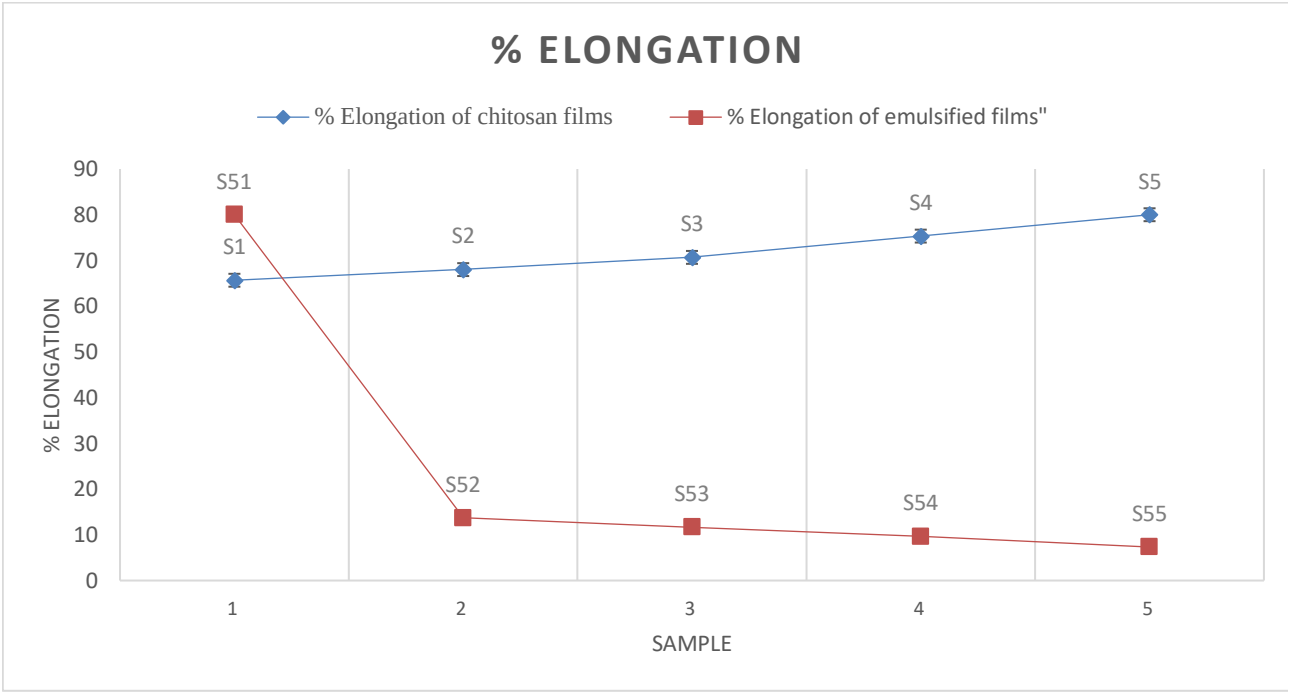


Figure 7 Comparison of % elongation of chitosan and emulsified edible films. Note: *Si = Aloe vera concentration; S5i = Beeswax concentration; S1 = 0%; S2 = 5%; S3 = 10%; S4 = 15%; S = 20%; S51 = 0%; S52 = 0.5%; S53 = 1.0%; S54 = 1.5%; S55 = 2.0%.

Water vapor permeability

Water vapor permeability chitosan films and beeswax films depended upon the *Aloe vera* and beeswax concentration in film forming chitosan respectively (Figure 4). Water vapor permeability was observed to be continuously decreasing as 0.044 $mgm^{-2}.h^{-1}.Pa^{-1}.mm$ at S1 to 0.012 $gm^{-2}.h^{-1}.Pa^{-1}.mm$ at S5 with increase in *Aloe vera* concentration in solution which was explained by **Ortega-Toro et al. (2017)**, **Khoshgozaran-Abras et al. (2012)** and **Elsabee and Abdou (2013)**. The higher water vapor permeability of the chitosan film could be due to its hydrophilic nature. It allows the water to come in interaction with matrix and results in increasing their rate of permeation **(Khoshgozaran-Abras et al., 2012)**.

Statistical analysis also proved that water vapor permeability significantly (p <0.05) reduced when the concentration of beeswax increased in the film forming solution. Chitosan-*Aloe vera* blend when mixed with beeswax produced hydrophobic effect in the films as lipid phase repels water **(Miranda et al., 2004)**. Water vapor permeability was observed to be reduced up to 81.12% when concentration of beeswax was increased from 0.5% to 2%.

Transparency

Transparency of edible films with respect to concentration of *Aloe vera* was statically analyzed and edible films was observed to be reduced 84.1% at S1 to 73.3% at S5 with incorporation of *Aloe vera* gel blend in film forming chitosan which was in agreement with **Pereira et al. (2011)**, **Khoshgozaran-Abras et al. (2012)**, and **Elsabee and Abdou (2013)**. Chitosan was observed to be transparent and decrease in transparency was due to incorporation of *Aloe vera* which contains phenols. Oxidation of phenols may be resulted in decrease in transparency of *Aloe vera*. Transparency of emulsified edible films with beeswax was also significantly affected (p <0.05). 43.1% reduction in transparency of emulsified films was observed from S52 to S55 **(Gomes de Santos et al., 2017)**. Chitosan films were observed to be transparent and decrease in transparency may be due to incorporation of *Aloe vera* and beeswax. Oxidation of phenols in *Aloe vera* as well as yellowish appearance of *Aloe vera* may be resulted in decrease in transparency of emulsified films (Figure 5).

Mechanical Properties

Means of tensile strength and percentage elongation at break of films was observed to be increasing with increase in *Aloe vera* concentration which was shown in the studies of **Pereira et al. (2011)**.

As the concertation of Aloe vera increased from S1 to S5, tensile strength and percent elongation at break was observed to be increased about 10.92% and 21.82% respectively. However, *Aloe vera* at 0% and 5% concentration was observed to have no significant effect (p < 0.05) on tensile strength as they are bearing same superscripts (Figure 6).

It was observed that tensile strength of emulsified films observed to be decreasing from 34.8 MPa at S52 to 26.5 MPa at S55 and percentage elongation was also reduced from 13.67% at S52 to 7.33% at S55. Comparing S51 with S52, S53, S54 and S55, a significant (p < 0.05) reduction in the % elongation was observed as 80% at S52 to 7.33% at S55 (Figure 7). The same trend was shown in the studes of **Velickova et al. (2013b)**.

Particle Size Distribution

Particle size of beeswax in *Aloe vera*-chitosan emulsion was tested with varying concentration of beeswax. These

emulsions were homogenized at 13,500 rpm for 1 min. No difference in particle size was observed when these films were subjected to particle size analysis. The films were tested considering D50 as standard which describe the particle size of 50% of the solution. The particle size of 50% film forming solution was observed to be 8.764 microns in all films.

Stability of emulsified edible films

Emulsified film forming solution prepared from *Aloe vera*-chitosan blend incorporating beeswax was tested for stability. 7 mL sample was measured and poured into the test tubes placed in test tube rack. These samples were kept for 3 days to check any separation in the emulsified film forming solution under ambient conditions. After 3 days, no change in the height and phase separation was observed in emulsified film forming solution.

CONCLUSION

A thin film of edible material is consumed and stops the transfer of oxygen, moisture, and the movement of solute from food. The edible films enrich the organoleptic characteristics of packaged foods as these films have several desired components (flavors, colors, sweeteners etc.). *Aloe vera* imparts antioxidant and antimicrobial effect in the biodegradable films. In order to control the diffusion rate of preserving agents from the surface to the interior of the food, these films are used on the food surface. Though, the permeability and mechanical characteristics of the edible films are usually inferior than synthetic films but, edible films reduce pollution and waste. In present study, edible films were, first, prepared using Chitosan and *Aloe vera* at different concentrations. The prepared edible films were then subjected to physical and mechanical analysis. The data obtained from various treatments was then analyzed using SAS 9.0 package. It was concluded that films with 20% *Aloe vera* had lower thickness, lower transparency, better mechanical properties and better water vapor permeability as compared to those with lower concentration of *Aloe vera*. Films with 20% *Aloe vera* were, then, selected and emulsified with beeswax in dispersed phase in Chitosan-*Aloe vera* solution at concentration upto 2.0% followed by film preparation through casting technique. The data obtained was again analyzed using SAS 9.0 package. The results revealed that thickness, tensile strength and water vapor permeability were increased when the concentration of beeswax increased from 0.5 to 2.0%. However, elongation at break and transparency was found to be decreasing with increase in beeswax concentration in the emulsified films. Further, no change in particle size was observed with change in concentration of beeswax. Insignificant difference ($p < 0.05$) was observed in the stability of emulsified blends after 24 hrs when stored at room temperature (25 °C, RH 70%). In addition, feasibility of these films was checked by calculating the cost on their development. Development cost of edible films found to be reasonable for high value and perishable products as an alternative of synthetic packaging.

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Contact Address:

*Usman Amin, University of Agriculture, Department of Food Engineering, University Road, Faisalabad, Pakistan, Zip Code 38000, Tel: +923047579504,
E-mail: Usman.amin@uaf.edu.pk
ORCID: https://orcid.org/0000-0002-5952-8243

Muhammad Azam Khan, University of Agriculture, Department of Farm Machinery and Power, University Road, Faisalabad, Pakistan, Zip Code 38000, Tel: +923004784972,
E-mail: uafkhan@yahoo.com
ORCID: https://orcid.org/0000-0002-1062-279X

Muhammad Ehtasham Akram, University of Agriculture, epartment of Food Engineering, University Road, Faisalabad, Pakistan. Zip Code 38000, Tel: +923216659359,
E-mail: ehtasham.akram@uaf.edu.pk
ORCID: https://orcid.org/0000-0001-9181-3071

Abdel Rahman Mohammad Said Al-Tawaha, Al-Hussein bin Talal University, Faculty of Science, Department of Biological Sciences, King Hussein Street, Postal Code 71111,, Maan, Jordan, Tel: 00962776693869,
E-mail: abdeltawaha74@gmail.com
ORCID: https://orcid.org/0000-0001-5726-4363

Alexey Laishevtcev, Federal Scientific Centre VIEV, Moscow, 109428, Russia, Orel State University named after I.S. Turgenev, Laboratory of Biological Control and Antimicrobial Resistance, 302026 Orel City, Russia, Tel: +7 (495) 970-03-68,
E-mail: a-laishevtsev@bk.ru
ORCID: https://orcid.org/0000-0002-5050-2274

Mohammad Ali Shariati, Orel State University Named After I.S. Turgenev, Laboratory of Biocontrol and Antimicrobial Resistance, 302026 Orel City, Russia, Tel: +7(4862)75-13-18,
E-mail: shariatymohammadali@gmail.com
ORCID: https://orcid.org/0000-0001-9376-5771

Corresponding author: *